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Page 12, line 13, *read* (1, p. 33, 37) *for* (1, p. 60); line 31, *insert* under "Cultural Characters": All the beef media used was made with *beef infusion* as a base, unless otherwise stated.

Page 21, *delete* reference 1 and *read* Rapp, C. W. Bacterial blight of beans. Okla. Agr. Exp. Sta. Bul. 131: 2–39. 1920.

Page 178, line 5 from bottom, *read* alsike clover *for* white clover.

Page 341, line 7 from bottom, *read* perithecia *for* pirithecia.

Page 385, line 2, *read* unsterilized *for* sterilized.

Page 423, footnote 2, line 2, *read* 1925 *for* 1924.

Page 518, legend for Fig. 2, line 2, *read* × 1000 *for* × 800.

Page 657, line 33, *read* teratological *for* teretological.

Cover of October number, *read* Albert S. Muller *for* Albert S. Miller.

Page 891, Table 1, column 3, *read* Cavangerie *for* Kavangerie.

Page 990, line 1, *read* it caused *for* is caused.

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BACTERIAL WILT OF BEANS (BACTERIUM FLACCUMFACIENS HEDGES), INCLUDING COMPARISONS WITH BACTERIUM PHASEOLI

FLORENCE HEDGES

INTRODUCTION

The disease described in this paper has been under observation by the writer since 1921, at which time navy bean plants were received from South Dakota accompanied by the statement that large numbers of the plants were dying. The wilted plants were found on the farm of the office of Forage Crop Investigations at Redfield, S. D., in navy beans which had been inoculated with *Bacillus radicicola*, and it was first thought by Mr. Leonard, of the office of Soil Bacteriology Investigations, that the nodule organism might have become pathogenic. This proved not to be the case. It was learned that the previous year there had been a loss of 90 per cent of the crop from what appeared to be the same disease, and that the 1921 crop was grown from seed harvested from the remaining 10 per cent. The loss in 1921 amounted to about 25 per cent. Some of the same seed planted at Arlington, Va., produced a large number of plants that wilted and died in the seedling stage. Examination of the plants from both South Dakota and Virginia showed bacteria in the vessels of the stem, often accompanied by browning. From diseased plants from both sources a yellow organism was isolated, with which the disease was repeatedly produced by artificial inoculation. This organism was found to differ from all bacteria hitherto described as infectious to bean, and was named *Bacterium flaccumfaciens*. A preliminary paper was published in Science (2), and a paper was given before the American Association for the Advancement of Science (3).

GEOGRAPHICAL DISTRIBUTION

The disease has been isolated from wilted navy bean plants from South Dakota, Michigan, Virginia and Maryland; and from seed from South

Dakota, Montana, Michigan, Maryland, District of Columbia, France and Germany. The author believes the disease is rather widely distributed in bean-growing sections but that it has been confused with the blight caused by *Bact. phaseoli*, and that in all probability the greater part, if not all, of the seedling wilt reported by the Plant Disease Survey of the Bureau of Plant Industry in Indiana, Michigan, Minnesota, New Jersey and Maryland in 1917, Minnesota and Connecticut in 1918, and New Jersey in 1920 was due to *Bact. flaccumfaciens*. In the summer of 1922 some Michigan fields showed as high as 90 per cent of the crop affected. It was the most serious disease affecting Michigan beans that season.

DESCRIPTION OF THE DISEASE

The following description applies to the wilt chiefly as it appears on the navy bean, inasmuch as this is the variety on which it has been most commonly observed and principally studied up to the present time.

The disease attacks all parts of the plant above ground. It may either kill the plant in the seedling stage, or dwarf it, resulting in a reduction in yield and the production of diseased seed.

Seedling wilt. Plants not more than 2 or 3 inches high may be attacked. The wilting and shrivelling of the leaves is sometimes accompanied by a dull green or brownish green discoloration, and the whole plant may be dead before it has developed more than the first pair of leaves.

Wilt of older plants. If the plant survives the seedling stage or becomes infected at a later period in its development, it may live throughout the season and mature its seed. When conditions are favorable, the diseased plants are very conspicuous because of the large number of shrivelled leaves (Fig. 1); but under conditions less conducive to severe attack they may be scarcely noted, as the wilting takes place slowly. Sometimes a seemingly vigorous plant bears one or more shrivelled lateral shoots more or less hidden by the mass of healthy foliage. Growers should be on the lookout for these less conspicuous signs, for such plants give the farmer little or no warning and yet produce diseased seed.

The discoloration accompanying the early stages of wilt when the leaves are becoming flabby is not, as a rule, very conspicuous. The color of the affected portion is dull green or brownish green, greenish brown, or reddish brown. The discolored area is flabby at first and later dry and papery. Now and then it is bordered by yellow, but this is not common. Sometimes the whole leaf blade and petiole become flabby and droop, unaccompanied by discoloration of any kind. Usually the wilting of a portion of the leaf is rapidly followed by the collapse of the whole, but occasionally the infection is restricted to a comparatively small area, the rest of the blade and the petiole remaining turgid at least for a time.

Another phase of the disease is a breaking over of diseased plants. In all cases observed, the break has occurred at a node which has been browned or reddened. The occurrence of this phase is probably dependent upon the amount and severity of wind and the degree of infection in the stem. It has been observed in the field only, never in greenhouse experiments even when the inoculation was made in a node. This phenomenon has been reported by Muncie (6) in connection with bacterial blight (*Bact. phaseoli*). From such broken over beans in Michigan in 1923, *Bact. flaccumfaciens* was isolated and its pathogenicity proved by inoculation experiments.

Pod infection. This disease is very much less conspicuous on the pods than bacterial blight (*Bact. phaseoli*). In fact sometimes, even on pods in which all the seeds are infected, there is no outward sign at all (Plate I, A and B) and on others it is so slight as to be easily overlooked. It does not consist of spotting as in blight, but infection takes place by way of the fibro-vascular bundles in the pedicel and manifests itself as a discoloration running along one or both sutures and sometimes spreading laterally to some extent. Infection may be observed on pods which are still green or just yellowing. On green pods the diseased area may be a rather sickly green or a yellowish green, or it may be somewhat darker than the rest of the pod and more or less watery looking. On ripe pods it is more conspicuous, the olive greenish color of the infected suture being then in greater contrast to the yellow color of the normal portion of the pod (Plate I, C). Infected pods have been found on large vigorous plants showing no other sign of the disease.

Seed infection. Diseased plants bear infected seed, but observations made thus far indicate that only a small proportion are visibly infected. There are two types of seed infection: (I) systemic or internal (Plate I, A), and (II) superficial. When seeds are harvested from an infected field, bright yellow seeds are very conspicuous among them. The infection is of type I and easily seen by the workers who hand-pick the seed in commercial seed houses. The culls from Michigan elevators in 1922 and 1923 contained large numbers of infected seeds and yet a sufficient number are overlooked, as shown by an examination of many packages of commercial seeds, to start the wilt in the fields the following year. The yellow color is caused by a thick bright yellow bacterial layer underneath the white seed-coat and visible through it. If the seed-coat is removed, this layer can be scraped up with the point of a knife, leaving the seed-coat white. Sufficient bacteria are present in a badly infected seed to make 10 cc. of water very milky. Sometimes the whole seed is yellow, sometimes only a portion, and sometimes it has a speckled appearance. Often such seeds are shrivelled, but they are quite as often plump and of normal size.

At times the infection is purely superficial (type II), bacterial slime being smeared on the surface of the seed from the interior of the pod, and sometimes the only sign of infection is a small amount of yellow slime at the hilum. Such seeds are sure to be overlooked by the farmer who saves his own seed or by the hand-pickers in the commercial seed houses. Unfortunately, the germination of even heavily infected seed does not seem to be greatly impaired, tests showing a germination of 72 per cent.

These tests were made with yellow seeds selected from culls from a Michigan elevator, so they were presumably from many different lots of seed and no checks were available.

Both types of seed infection may occur in cases of bacterial blight (*Bact. phaseoli*) also, and when they do occur can not be differentiated by the eye alone from those due to bacterial wilt (*Bact. flaccumfaciens*).

The writer believes, however, that type I (systemic or internal infection) is rare in cases of bacterial blight (*Bact. phaseoli*), because systemic infection of the plant as a whole is rare in that disease, though it does occur. Furthermore, there is the circumstantial evidence that but three isolations from 18 samples of diseased seed (type I), from as many sources, yielded the blight organism (*Bact. phaseoli*), whereas the other fifteen platings gave pure cultures of *Bact. flaccumfaciens*, the wilt organism, regardless of the fact that the bacterial blight (*Bact. phaseoli*) occurs very commonly in all bean-growing sections.

The writer is of the opinion, from observations made up to the present time, that the greater part of the yellow seed answering the above description is infected with the wilt (*Bact. flaccumfaciens*) instead of the blight organism (*Bact. phaseoli*) and that these two diseases have been confused on the seed as well as in the field.

HOST PLANTS

The disease has been found occurring naturally on navy, wax and lima beans and has been produced artificially in soy beans (Var. Ito San). Tests of other legumes and related plants are contemplated.

PLANTING OF DISEASED SEED

On May 20, 1922, seed from the diseased crops grown in 1920 and 1921 at Redfield, S. Dak., was planted at Tuxedo, Md., on land on which beans had never before been grown. Yellow seed from two other lots, bought on the market in Baltimore and Washington, and known to contain *Bact. flaccumfaciens*, also were planted in this plot, altogether about 28½ oz. of seed. Unfortunately, the writer was unable to obtain, that season, any commercial samples of navy bean seed wholly free from wilt and accordingly was

obliged to use for the check plot seed selected with as much care as possible from lots known to be infected.

Two weeks after planting, when the plants were about 2 inches high, most of them had developed the first compound leaf and there was 2.2 per cent wilt. No wilt had developed in the check plot.

Examinations were made of the plot from time to time during the summer, and an increased number of wilted plants was found on each successive examination.

The results of the experiment were inconclusive because of the impossibility of obtaining good seed for checks and because the plants in two whole rows, including the checks, became badly infected with mosaic. Considerable blight (*Bact. phascoli*) also was present.

On August 28 the crop was harvested. The plants were much stunted and had produced very few pods. Of the 15 per cent which still bore living shoots, 88 per cent were examined microscopically and 70 per cent were found to contain bacteria in the stem. The plants bore very few pods, and many of these were infected with *Bact. flaccumfaciens*. Many cases of wilt occurred among the checks also, a result which was not surprising. The experiment would not have been worth recording had it not been that no beans had ever been grown on this ground before and there were none in the vicinity from which the disease might have been carried.

Wilt also occurred on plants grown from diseased seed in sterilized soil in the greenhouse (Plate II, E).

FIELD OBSERVATIONS

Arlington, Va. The writer first saw this disease in the field at the Arlington Experiment Farm, Va., Aug. 30, 1921, in an experimental plot belonging to Mr. Leonard, of the office of Soil Bacteriology Investigations. Mr. Leonard had undertaken to determine what influence, if any, the treatment of bean seed incidental to its inoculation with liquid cultures of *Bacillus radicicola* might have upon bean wilt (5), that disease having been first observed in conjunction with inoculation with the root nodule organism at Redfield, S. D. Seed from the diseased Redfield crop and from the markets in Baltimore and Washington, D. C., was used. Wilt occurred in plants from all three lots, but the writer's observations were concerned chiefly with the progeny of the Redfield seed.

The plants were in the seedling stage, and great numbers of them had wilted and died. The beans were planted in hills, and in some cases as many as five plants in seven were wilted. Ten partially wilted plants were taken up and planted in pots in the greenhouse. For a time they continued to grow slowly, the new leaves wilting shortly after unfolding, with the

exception of one plant more resistant than the rest. In 5 days, 5 of the seedlings had no turgid leaves and 5 had one each. In 12 days, 5 were dead; and in 6 weeks, 7 of the 10 were dead and 2 nearly so. The remaining plant had been much more resistant. It had grown more rapidly than the others and, whereas, on the 5th day it had only one turgid leaf, on the 12th day it had 3 and on the 22nd day it still had two which showed no sign of wilt. At the end of 6 weeks the last leaf of this most resistant plant of the ten was flabby, and the plant finally succumbed to the disease.

A final examination was made of the Arlington plot on Oct. 10. The majority of the plants were very much stunted, the leaves were abnormally small and many of them were flabby or dead. The tips of many small lateral shoots were wilted and shrivelled. Many plants were entirely dead and few appeared to be normal.

Michigan. In the summer of 1922, Dr. R. D. Rands, of the Bureau of Plant Industry, made an extended survey of the bean-growing districts in Michigan and reported the occurrence of much wilt there, stating that it was the most serious bean disease in the state that season. Infected plants collected by him were turned over to the writer, who isolated very virulent strains of *Bact. flaccumfaciens* from the seeds. The following summer (1923) the writer visited several of the largest bean-growing centers in the state during the latter part of June and of August. Because of the prevalence of the wilt the preceding year, it seemed probable that a considerable amount of infected seed would find its way into the fields at planting time and that seedling wilt would result. Such proved to be the case, although only one field was found where the wilt was present in quantity at the time of the first visit in June. This was a 10-acre field of Robust bean and it was badly infected with seedling wilt, although no dead plants were observed, and most of the plants had only one or two wilting leaves. An examination of the seed left after the planting of this field showed a considerable proportion of infected seed.

On this tour of inspection infection was found on both Robust and Improved Robust, two varieties of navy bean widely grown and highly prized in Michigan because of their resistance to disease. Early Wonder also was found to be attacked. In several instances where wilt occurred the writer had an opportunity to examine the seed left from the planting, and in every case it was found to be infected. Infected seed was very common in a sample examined at one of the elevators in Tuscola County, the county in which the greatest amount of wilt was found.

In August the same fields were re-visited, and wherever seedling wilt had been observed in June, infected seed was found in August. Infected pods and seed were very plentiful in the field previously mentioned, in

which so much seedling wilt was seen in the spring, although, owing to the excessively dry weather, no doubt, it had been so inconspicuous during the summer that the owner of the field had believed his plants had outgrown the disease and was much amazed when the infected pods were pointed out to him. He had been greatly interested in the writer's photographs and colored plates of the wilt on the previous visit and had been on the lookout

FIG. 1.—Wilted navy bean plant from badly infested field, Redfield, S. D. Courtesy of L. T. Leonard, U. S. Dept. of Agr.

for the disease during the summer. This is an instance of the manner in which, under certain conditions, the wilt can persist in a field and produce diseased seed without attracting notice. Although there had been much seedling wilt in the spring, the disease had progressed so slowly during the dry season that few plants had wholly succumbed, and the wilted leaves had been so few at any one time and dropped off so gradually that the field appeared to be free from the disease. This was in marked contrast to the Redfield, S. D., field in 1920 and 1921 and fields in Michigan the previous year (1922) as reported by Leonard and Rands, respectively. In these fields badly wilted plants (fig. 1) were of common occurrence.

In one Michigan field of Early Wonder in which but little seedling wilt was observed in June, there was a great quantity of pod and seed infection (Plate I, C). This field also had been planted with infected seed, as shown by examination of the seed left after the planting.

The breaking over of the stems near the ground was observed in August in one of the experimental plots at the Michigan Agricultural College in which the plants were badly wilted. Nearly all of the plants affected with wilt were broken in this manner. From such plants, *Bact. flaccumfaciens* was isolated. The breaking was usually accompanied by browning or reddening of the stem.

ISOLATION OF THE ORGANISM

The organism has been isolated in pure cultures repeatedly from stems, petioles, and leaf blades of both seedlings and older plants, and from the seed. The colonies appeared, as a rule, in two days. When isolations were made from stems, petioles or leaf blades, the tissue was dipped in alcohol, sterilized in mercuric chloride 1 : 1,000 for twenty seconds to two minutes. Leaf blades were never sterilized more than one minute. The mercuric chloride was then washed off in sterile water, the tissue transferred to beef bouillon or another tube of sterile water and crushed. From the tube containing the crushed tissue, dilutions were made and plates poured.

The organism is easily obtained from the seed in pure cultures without sterilization, both from the bacterial layer under the seed coat and from dried bacterial ooze on the surface. In the case of the former the seed was split, the seedcoat with its bacterial lining removed, dropped into sterile water and left standing a few minutes before the dilutions were made, in order that the bacteria might diffuse. When pouring plates from dried bacterial ooze on the surface of the seed, the whole seed was dropped into sterile water.

The organism is most surprisingly resistant to drying, as is evidenced by the fact that it has been isolated from dried bacterial ooze on the hilum

of seed nearly 5 years old. Even in this case the colonies appeared in 2 days and were just as virulent as isolations from the most recent infections.

INOCULATION EXPERIMENTS

Prick inoculations. Artificial infection has been repeatedly produced by pricking bacterial slime from pure cultures of *Bact. flaccumfaciens* into stems, petioles, or pods of sound plants. Sometimes the plants were put into inoculation cages but this is not necessary. The plants were, however, kept shaded for the first 12 or 24 hours after inoculation. Plants of all ages were inoculated in the stem—from tiny seedlings in which the cotyledons had not yet spread apart to those which were in blossom. The plants are susceptible at all ages if thrifty and growing well. Older plants were usually inoculated near the tip of the main stem or on a lateral shoot, although infection also can be obtained by inoculation near the base of the plant. The first signs of infection usually were observed in 5 to 10 days, although in one experiment wilting occurred in 3 days and in another the first signs of infection were observed in 2 weeks. In one set of inoculations there was 100 per cent infection in 11 days.

The wilting and shrivelling of the first leaf above the pricks was followed by the successive wilting and falling of the leaves above until the stem was defoliated or nearly so (Plate II, A–C). Sometimes the plants were completely defoliated in 14–18 days. At other times the disease progressed much more slowly. If the plants lived long enough to produce pods, these were commonly infected (Plate II, A).

In some of the artificial infections, yellowing occurred. This was quite common in two of the inoculation experiments, one made Dec. 20, 1921, on very young King of the Garden Lima beans and Ito San soy bean seedlings, the other made Oct. 24, 1922, on navy beans 1–2 feet high. Pure cultures of *Bact. flaccumfaciens* were repeatedly obtained by plating from these yellow areas, which appeared in 13–39 days. This phenomenon, however, has been observed too rarely to be considered one of the characteristics of the disease.

Another very successful method of obtaining infection on very young plants was by removing the cotyledons and painting the wound with a brush dipped in a water suspension of *Bact. flaccumfaciens.*

A simple time-saving device was constructed for use when large numbers of plants were to be inoculated, consisting of a small camel's hair brush to which a fine needle had been bound. The point of the needle was about 2 mm. below the end of the brush. With this device the wounding and application of the bacteria were effected at the same time. Since the instrument could not be flamed, as many of them were used as there were colonies concerned in the inoculation.

Very interesting and unexpected results were obtained when prick inoculations were made on the green pods. The Lazy Wife pole bean was the variety used in every case and inoculations were also made with *Bact. phaseoli* for comparison. There was so little reaction on the surface of the pods inoculated with *Bact. flaccumfaciens* in 10–12 days that the writer thought no infection had taken place. In 14–16 days, however, the pods were withering and in some cases yellowing. At this stage they were opened and the seeds were found to be badly infected (Plate III, A). Where the needle pricks had gone into the seeds, there was a bright yellow bacterial layer under the seedcoat. There was also considerable bacterial slime on the inner surface of the pod and in one case the latter was discolored. The interior of the pods inoculated with *Bact. phaseoli* presented an appearance very similar to that just described, but the exterior showed a decided contrast, the pricks in the inoculations with *Bact. phaseoli* being surrounded by water soaking, which appeared in 7 days and left no doubt as to the success of the inoculation.

Prick inoculations also were made on the pedicels and on the stem just below the pedicel. In 3 weeks, infection was visible along the seed-bearing suture, which was darker green than the rest of the pod and in some cases was splitting. There was also bacterial ooze in some instances. In 4 weeks the pods were beginning to wither and were turning a yellowish green in the flabby portion (Plate III, B). The pods were opened and found to contain infected seed. There was conspicuous watersoaking in the pods similarly inoculated with *Bact. phaseoli*, a marked contrast to the preceding. The infection had likewise entered the seeds, which resembled those infected with *Bact. flaccumfaciens*.

All attempts made to infect flowers failed, as in every case they fell off. When extremely young pods were inoculated, they either fell off or failed to mature.

Applying a bacterial suspension of *Bact. flaccumfaciens* to the uninjured surface of green pods with a brush produced no infection, but a similar treatment with *Bact. phaseoli* produced typical watersoaked spots.

Spray inoculations. None of the writer's experiments have given conclusive evidence of the occurrence of stomatal infections and it is the opinion of the author that they do not take place. They certainly do not do so readily, as is the case with *Bact. phaseoli*. Spray inoculations have been made on seedlings of navy beans, King of the Garden Lima and Ito San soy bean, with prick inoculations as checks. The plants were kept in a moist atmosphere at least 24 hours. In one set of these experiments a method was used which had proved very effective in producing infection in soy beans with *Bact. phaseoli*, a difficult accomplishment, *viz.*, spraying

seedlings a few days old in the damp chamber in which they had been germinated (4). Three days later the sprayed seedlings were planted in pots in the greenhouse. The prick inoculations resulted in infection in 5 to 13 days, but whenever infection occurred on the sprayed plants it was on a torn leaf or on a plant which had been so gnawed by insects that it seemed probable that the bacteria had entered through the wounds. In some instances it was very evident that this was the case. No small spots, gradually increasing in size indicative of stomatal infections, were ever observed.

On the other hand, that the bacteria easily can find their way into wounds without being forced in mechanically as in the case of the needle-prick inoculations described above, is evident from the fact that infection readily took place on King of the Garden Lima beans when the plants were pricked first and then sprayed with a water suspension of the bacteria. The plants were kept moist in inoculating cages for 46 hours. Local infection occurred on Ito San soy bean leaves which had been rubbed between the thumb and forefinger after spraying, but no wilt resulted. The infection was confined to brown streaks on the leaves from which *Bact. flaccumfaciens* was re-isolated. On the other hand, this plant succumbs readily to prick inoculations.

Infection was obtained upon all the varieties tested, *viz.*: Great Northern Navy, Common Navy, King of the Mountain Navy, Black Valentine, Lady Washington, Early Wonder, Red Kidney, Wardwell's Kidney Wax, Davis White Wax, Vaughan's Stringless Wax, Lazy Wife, King of the Garden Lima and Ito San soy bean.

The colonies used for the inoculations came from widely separated sources: South Dakota, Montana, Michigan, Virginia, Maryland, France and Germany. All were equally virulent.

SUSCEPTIBLE VARIETIES

The disease has been found occurring naturally on the following varieties: Common Navy, Early Navy, Great Northern Navy, Improved Navy, Navy Pea Bean, Pea Bean, Pilot Navy, Black Valentine, Crystal White Wax, Early Wonder, Golden Eye Wax, Henderson's Bush Lima, H. trés nain précoce, Hinrichs Riesen, Improved Robust, Robust, S.P.I. 50892, S.P.I. 50894, Wardwell's Kidney Wax, White Kidney, and a pink seeded variety from Michigan.

Wardwell's Kidney Wax was reported as being especially susceptible to wilt in Michigan in 1922.

In addition to the above, the following were found by inoculation to be susceptible: Davis White Wax, King of the Garden Lima, King of the

Mountain Navy, Lady Washington, Lazy Wife, Red Kidney, Vaughan's Monster Stringless Wax and Ito San soy bean.

Variety tests on a large scale have been carried out with the wilt organism by Rands and Brotherton (7) of the Bureau of Plant Industry in connection with parallel experiments on mosaic, anthracnose and bacterial blight (*Bact. phaseoli*), with a view to developing varieties resistant to these enemies of the bean crop. No variety wholly immune to *Bact. flaccumfaciens*, however, has been found.

CONTROL METHODS

The use of clean seed from wilt-free fields is the best means of control thus far known, both observation and experiment having shown that this disease is transmitted through the seed. The use of seed two or three years old as recommended by Rapp (1, p. 60) of the Oklahoma Agricultural Experiment Station, as a control for *Bact. phaseoli* would avail nothing in this case, as the causal organism of wilt has been found alive and virulent on seed five years old. It is doubtful whether any form of seed treatment would be practicable, as the bacteria are underneath the seed coat and protected by it. The ease with which the seed coats slip would preclude any long treatment with liquids, and the thermal death point of *Bact. flaccumfaciens* is so high (60° C.) that a temperature which would kill it would probably kill the embryo of the seed. However, experiments along this line are underway.

Until more is known about the possibility of infection through the soil, it is advisable not to use bean straw as fertilizer or feed. Experiments on soil infection have been undertaken.

Leonard (5) states that in his experiments in South Dakota there were indications of a stimulation of the wilt disease when bean seed was inoculated with the root-nodule organism by means of liquid cultures. He suggests the applications of *Bact. radicicola* in the form of inoculated soil applied in a dry condition.

CULTURAL CHARACTERS

Colonies on 1 per cent peptone beef agar plates.—Appear usually in 2 days, whitish at first but very soon becoming pale yellow or Ridgway's cream color (8). They are round, smooth, wet-shining, semi-opaque, with entire, slightly thinner margins, flat or slightly convex, coalesce readily, and are finely granular. On very thinly sown plates they may become 4–7 mm. in diameter. A small brighter yellow type of colony occasionally appears on the plates but this appears to differ in no other respect from the pale

yellow or cream colored type either on other culture media or in its host reactions. No internal markings.

Beef peptone agar slants.—Moderate flat spreading, pale yellow growth in two days at 22° C.: glistening, slightly contoured, translucent and viscid. It is sometimes difficult to remove the slime from the agar and difficult to make it diffuse in water.

Beef peptone agar stabs.—Good pale yellow to deep yellow nailhead covering the surface of the agar. Scanty growth in the stab, very rarely any below 1 cm. in depth.

Beef peptone gelatin stabs.—Very slow liquefaction. Little or no liquid gelatin present during the first month, evaporation taking place almost as rapidly as the liquefaction in contrast to the rapid liquefaction by *Bact. phaseoli.* In 9 weeks the gelatin was 1/3 to 1/2 liquefied.

Congo Red agar (Kellerman).[1]—Very thin, scanty colony-wise growth, colorless at first but later absorbing the Congo red. The agar becomes purplish in 2–4 weeks. An excellent medium for distinguishing *Bact. flaccumfaciens* from *Bact. phaseoli.* The latter makes a copious smooth wet-shining growth in decided contrast to the meager growth of the former.

Lima bean agar.—Very scanty growth, thin, slightly rough, pale yellow.

Litmus lima bean agar.—A very scanty growth bluing the litmus.

String bean agar.—Thin, dull, finely roughened growth, colorless at first but becoming yellowish. An excellent medium for differentiating this organism from *Bact. phaseoli* which makes a good wet-shining growth upon it.

Soil agar (Löhnis).[2]—Very scanty, barely visible growth.

Starch agar.—Very weak diastasic action on cornstarch beef infusion agar. Plates tested with iodine when 1 week old. An excellent means of differentiating *Bact. flaccumfaciens* from *Bact. phaseoli* (Fig. 2).

[1] Distilled H_2O 1,000.00 c.c.
Saccharose 10.00 g.
Dipotassium phosphate 1.00 g.
Magnesium sulphate 0.20 g.
Agar flour 15.00 g.
Congo red 0.10 g.
Steam water, agar and salts one-half hour then add Congo red.

[2] Soil 150 g.
Distilled H_2O 1,000 c.c.
Calcium oxide trace
Heat in steamer 1 hour, filter and add per 1,000 c.c.:
Dipotassium phosphate 0.5 g.
Mannite 10.0 g.
Agar flour 15.0 g.
$CaCO_3$ excess.

Thaxter's potato agar.—Good but not copious, whitish growth, somewhat contoured but for the most part smooth. Dry shining. Excellent medium for differentiating this organism from *Bact. phaseoli*, which makes thereon a copious wet-shining yellow growth.

Whey agar.—Good but not copious growth, dry-shining and somewhat contoured. When 10 days old the bacterial layer varied from primuline yellow to buff or apricot yellow (8). A marked contrast to the copious smooth wet-shining growth of *Bact. phaseoli* on this medium.

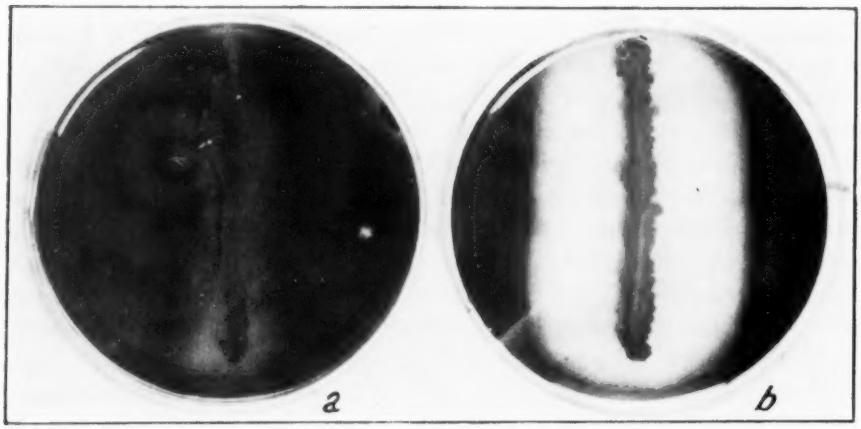

FIG. 2.—Cornstarch beef infusion agar plates 7 days old tested with iodine, showing comparative amount of diastasic action. × ½ ca.

A. *Bact. flaccumfaciens.* Weak diastasic action.

B. *Bact. phaseoli.* Strong diastasic action.

Yeast agar + 1 per cent dextrose.[3]—A very good medium for this organism. There is a thin, whitish colony-wise growth in 24 hours if transferred from young cultures. Excellent whitish or cream-colored, wet-shining, somewhat contoured growth in 3 to 6 days. Color deepens to primuline yellow (8), in old cultures (4–5 weeks) and the bacterial layer becomes smooth.

Potato.—Moderate, often scanty mustard or primuline yellow (8) growth usually accompanied by a marked graying of the potato; very little diastasic action; a striking contrast to the copious growth and strong diastasic action of *Bact. phaseoli.* Growth on potato is extremely variable, sometimes ranging from little or none to very good growth in the same series

[3] 200 grams yeast (Red Star yeast, free from starch is recommended), 2,000 c.c. tap water. Mix thoroughly and heat in steamer for 2–3 hours. Stopper with cotton and autoclave for at least ½ hour. Let stand several days. Siphon off the top liquid, adjust reaction, add agar and sugar.

of cultures of the same colony, probably due to differences in the individual potatoes.

Beef infusion peptone bouillon.—Thin to moderate clouding within 24 hours, often noticeably more clouded at the top, at temperatures ranging from 20° to 36° C. The clouding may increase somewhat and is persistent at favorable temperatures. A partial or complete pellicle usually forms in 2 or 3 days. The character of this varies considerably. When young it shakes down easily, either falling as a whole, breaking up into coarse flocculence or falling in long strings when agitated slightly. In cultures two weeks old which have not been disturbed the pellicle may be thick, exceedingly viscid and difficult to shake down. Sometimes a heavy rim is formed, but many cultures form only a faint partial one. The formation of pseudozoogloea is also an inconstant character. When present, they are sometimes more numerous in the upper portion of the medium.

A very viscid ropy precipitate forms, rising in swirls when shaken and sometimes detached from the bottom of the tube only with the most vigorous shaking. No odor.

Cohn's solution.—Little, if any, growth.

Fermi's solution.—Some colonies cloud this medium very faintly, others not at all. Clouding when it does occur is transient.

Litmus milk.—A slight alkalinity often occurs the first few days. Litmus reduced in 4–12 days. Acid reaction in 3–4 weeks. Other changes as in milk *q. v.*

Milk.—A solid acid curd is formed in milk, although cultures are often slightly alkaline the first few days (compare lactose agar). Coagulation normally begins in 3 or 4 weeks, occasionally in 2 weeks, and on the other hand, sometimes in a series of cultures one which has remained fluid long after the others, will coagulate ultimately.

One to several days after the formation of the solid curd there is an extrusion of whey and shortly thereafter peptonization begins, progressing very slowly. Rare cases have been observed in which peptonization began in 2 weeks without the forming of a solid curd. Tyrosin crystals have been observed in cultures 3 months old. *Bact. flaccumfaciens* usually produces a wide deep, primuline yellow rim in milk which is very striking.

This is a good medium for differentiating this organism from *Bact. phascoli.*

Uschinsky's solution.—Thin to moderate clouding. No rim, pellicle or pseudozoogloeae.

FERMENTATION

Fermentation tubes.—No gas was produced in fermentation tubes containing beef extract broth with brom cresol purple, 1 per cent peptone and

1 per cent dextrose, lactose and saccharose. Little, if any, clouding in the closed end. Acid reaction in all, visible in saccharose in 18 to 42 hours, in dextrose in 42 hours, and in lactose in 5 to 7 days. Temperature 21–26° C. Alkali was sometimes produced in lactose following the production of acid. In dextrose and saccharose the reaction was still acid after 2 months.

Litmus lactose agar (1 per cent beef infusion agar + 1 per cent peptone + 1 per cent lactose).—Pinkish cream colored growth. Trace of reddening (10 days). Reduction of the litmus. Good medium for differentiating *Bact. flaccumfaciens* from *Bact. phaseoli* when cultures are young. Old cultures (2 mos.) of both organisms are bluish green.

Sugar and glycerine agars with brom cresol purple and phenol red (slant beef extract agar + 1 per cent peptone and 1 per cent of the substance to be tested). There was good growth in all. No gas was produced. *Bact. flaccumfaciens* was yellow on the media containing brom cresol purple but nearly white on that containing phenol red. The cultures were kept at room temperatures which varied considerably during the course of the experiments (21° C. to 32° C.). The results were as follows:

Dextrose.—Acid produced in 2 days. Cultures still acid after 2 months with the exception of one colony which began to show an alkaline reaction in 17 days.

Lactose.—Slight but distinct alkaline reaction in 2 days in most cases. Acid produced in 3–5 days. Occasionally a colony did not show the initial alkalinity preceding acidity. The cultures finally became alkaline, usually in 12–19 days. One culture, however, which remained acid for 9 weeks gave an alkaline reaction in 11 weeks.

Saccharose.—Acid reaction in 18 to 48 hours. With two exceptions the cultures remained acid for 2 months. Two colonies, however, became alkaline in 2 and 6 weeks.

Glycerine.—Acid reaction in 2 days. All except one colony, which showed a trace of alkali in 10 days, remained acid for 2 months. The culture of the exceptional colony was alkaline throughout in 30 days.

These sugar and glycerine agars afford a very excellent means of distinguishing *Bact. flaccumfaciens* from *Bact. phaseoli*, as cultures of the latter produce no acid but become alkaline in 2 to 3 days.

PRODUCTION OF AMMONIA, HYDROGEN SULFIDE AND INDOL

Ammonia was present in beef extract peptone broth (pH 7.0), tested with Nessler's solution 5 days after inoculation.

No hydrogen sulfide was formed in basic lead acetate beef extract agar cultures under observation for 3 weeks.

No indol present in Uschinsky + peptone cultures tested the 5th day with sodium nitrite and sulphuric acid. *B. coli* was used as a check.

REDUCTION OF NITRATES TO NITRITES

No reduction of nitrates in nitrate-peptone beef extract broth or synthetic nitrate solution in 2, 4, 5 or 11 days. Ammonia was produced in both the nitrate and the plain beef broth. The organism had not used the potassium nitrite in the peptone water + 2 p. p. m. potassium nitrite. Temperature 26° to 32° C. Sulphanilic acid and α-naphthylamine test used. Negative result also obtained with the potato starch water-potassium iodide-sulfuric acid test. No gas formed.

In earlier experiments the cultures were put into the thermostat at 32° C., which is about the optimum temperature for this organism. The long continued heat broke down the nitrate and in 7 days both the cultures and the uninoculated nitrate broth checks gave the nitrite reaction. This was confirmed by later experiments in which parallel sets of nitrate broth were inoculated, one being kept at room temperature, the other in the thermostat. The temperature of both places varied considerably during the experiment—the thermostat from 31° to 35° C., room temperature from 21° to 29.9° C., but when tested with sulphanilic acid and α-naphthylamine in 8 days all those in the thermostat, including the uninoculated checks, gave the deep pink nitrite reaction and all at room temperature remained colorless. *B. phytophthorus* was used as a check.

TOLERATION OF ACID

Tests were made in 17 beef infusion peptone bouillons ranging from pH 9.1 to pH 4.9. The optimum pH proved to be 8.0 to 6.2, clouding taking place in 9½ hours. The maximum tolerated acidity was about pH 4.9, some of the tubes clouding, others remaining clear. Growth occurred in pH 8.6 bouillons in two days, and some of the tubes clouded in the pH 9.1 bouillon in 5 days, at which time it had become pH 8.84.

TOLERATION OF NaCl

Growth occurred in beef bouillon containing 2, 3, 4 and 5 per cent NaCl. All but the 5 per cent had clouded in 42 hours. The 5 per cent sodium chloride produced quite an inhibitory effect, but in 5 days there was slight growth and transfers made from these cultures to beef broth produced clouding in 24 hours, showing that the vitality of the organism was unimpaired. Cultures were kept in the thermostat at 29°–31° C.

EFFECT OF FREEZING

Beef broth transfers from 24 hour old beef broth cultures were frozen in salt and ice for 15 minutes, 40 minutes and 1 hour, then thawed quickly and kept at room temperature (24° C.). All were clouded in less than 24

hours, though those exposed for an hour were less clouded than the checks.

LONGEVITY

Cultures have lived for 4 months at 1.5° to 2° C. Beef broth cultures at winter room temperatures (about 22° to 24° C.) were alive after 7 weeks, but cultures of the same colonies transferred at the same time from the same source to Thaxter's potato agar were dead at the end of this period.

The organism has been found alive and virulent on the seed, both under the seedcoat and on the exterior, at the end of five years, as already stated.

REACTIONS TO STAINS

Bact. flaccumfaciens stains readily with carbol fuchsin and Ehrlich's anilin gentian violet. It is Gram positive[4] (unlike *Bact. phaseoli*) and not acid fast. No capsules were demonstrated with Ribbert's capsule stain, although covers were stained from very viscid growth on 48 hour old beef agar slants, and from very viscid nitrate beef broth cultures.

SUNLIGHT TESTS

The only sunlight tests were made in January at Washington, D. C., on bright, cloudless days. Plates poured from beef broth cultures 24 hours old were exposed bottom side up. They were placed on ice and so slanted as to catch the direct rays of the sun. Exposures were from 30 minutes to 2 hours in length and in none of them were all the colonies killed. Half were killed in 30 minutes, two-thirds by 45 minutes and very few more by 2 hours. The 2 hour exposure, however, was later in the afternoon.

TEMPERATURE RELATIONS

Thermal death point.—The thermal death point is about 60° C. The majority of the cultures tested were killed by 57.5° and 59.9° C. but one colony survived both these temperatures. Beef bouillon tubes inoculated with 24 hour old beef broth cultures were exposed for 10 minutes in the water bath, readings being taken every half minute.

Maximum, minimum and optimum temperatures.—The minimum temperature is below 1.5° C. Tubes subjected to a temperature ranging from 0.9° C. to 1.5° C. remained clear for 2 weeks but became faintly clouded after 7 weeks. Others placed at a temperature ranging from 1.5° C. to 2° C. were clouded in 6 to 13 days. These cultures were alive at the end of 4 months.

The maximum temperature is between 36° C. and 40° C. In the thermostat, ranging from 38.5° C. to 40.5° C., there was no growth.

[4] *B. phytophthous* (Gram negative) used as check.

The optimum temperature is about 31° C. Growth occurred in less than 24 hours at 20°, 23.5°, 24°, 26°, 28°, 30.8° to 31.5°, 32° and 35.5° to 36° C., the most clouding at the end of this period being at 30.8° to 31.5° C. and the least at 35.5° to 36° C.

For these tests, peptonized beef broth was inoculated from peptonized beef broth cultures 24 to 48 hours old.

TABLE 1.—*Tests for differentiating Bact. flaccumfaciens from Bact. phascoli*

Medium	*Bact. flaccumfaciens*	*Bact. phascoli*
Congo Red agar	Very thin, scanty colony-wise growth	Copious, smooth, wet-shining growth
Cornstarch agar	Very weak diastasic action	Very strong diastasic action (Fig. 2)
Gelatin	Very slow liquefaction	Rapid liquefaction
Glycerine beef extract agar with brom cresol purple and phenol red	Acid reaction in 2 days	Alkaline reaction in 1–3 days
Litmus lactose agar	Pinkish cream growth: reduction and no bluing of litmus	Greenish yellow growth: bluing and no reduction of litmus
Milk	Slow formation of solid acid curd followed by separation of the whey and slow peptonization	Clearing 1–6 days; soft mobile curd, rapid peptonization preventing formation of a solid curd. Secretes a lab ferment (EFS)
Potato	Scanty to moderate growth accompanied by marked graying of the cylinder: very weak diastasic action	Very copious growth on fresh potato cylinders, finally so filling the water with slime that it remains in position when the tubes are inverted. Very strong diastasic action
String bean agar	Thin dull finely contoured growth	Good wet-shining growth
Sugar beef extract agars with brom cresol purple and phenol red. (dextrose, lactose, saccharose)	Acid reaction in 2–3 days	Alkaline reaction in 2–3 days
Thaxter's potato agar	Good but not copious, dry-shining, whitish, somewhat contoured growth	Copious wet-shining yellow growth
Whey agar	Good but not copious dry-shining somewhat contoured growth	Copious smooth, wet-shining growth
Gram's stain	Positive	Negative (EFS)

TECHNICAL DESCRIPTION

Bacterium flaccumfaciens Hedges.

Short, non-spore-forming rod with rounded ends, 0.6 to 3 μ x 0.3 to 0.5 μ, occurring singly or in pairs. No chains or filaments have been observed. Yellow (sometimes pale cream color), motile by means of a single polar flagellum 3 or 4 times the length of the rod, stained from 24 hour old beef agar slants with Casares-Gil's stain; aerobic; Gram positive, not acid fast, stains readily with carbol fuchsin and anilin gentian violet; produces neither nitrite nor gas in nitrate media; liquefies gelatin slowly; weak diastasic action; produces acid without gas from dextrose, lactose, saccharose, and glycerine; no capsules demonstrated; on beef agar slants produces moderate, flat, glistening, slightly contoured, translucent, cream to yellow, viscid, odorless growth; agar colonies round, smooth, wet-shining, flat, semi-opaque, finely granular with entire margins, appearing usually in 2 days; acid curdling and peptonization of milk and production of tyrosin crystals in old milk cultures; reduction of litmus in litmus milk; moderate to strong persistent clouding of nutrient broth, odor absent, sediment viscid on agitation; produces ammonia in beef broth + peptone; no production of indol in Uschinsky + peptone (5 days); no hydrogen sulfide produced in basic lead acetate agar; little, if any, clouding of the closed end of fermentation tubes containing peptonized beef extract broth containing dextrose, saccharose and lactose. Pathogenic to beans, causing dwarfing and wilt of seedlings and older plants and infection of the seed through which it transmits the disease.

For the sake of facilitating the diagnosis of *Bact. flaccumfaciens* a brief table of cultural tests is appended which will serve to differentiate it from *Bact. phaseoli* with which it has been confused in the past (Table 1.)

SUMMARY

This paper describes a seed-borne bacterial disease of beans causing wilting and death of seedlings, and wilting, dwarfing and sometimes breaking over of older plants. It is known to occur in South Dakota, Michigan, Montana, Virginia, Maryland, France and Germany.

The causal organism, *Bacterium flaccumfaciens* Hedges, has been isolated and infection repeatedly obtained through pure culture prick inoculations with subcultures from single colonies. Stomatal infections common in bean blight (*Bact. phaseoli*), do not occur.

Artificial infection also has been produced upon soy bean, but the disease is not known to occur naturally upon this crop.

Bean wilt is much less noticeable upon the pods than is bacterial blight (*Bact. phaseoli*) and might be easily overlooked, but the bright yellow

infected seeds of white seeded varieties are very conspicuous. They are common even in hand picked commercial seed following a season in which much wilt has occurred.

Observation and experiment have shown that the disease is transmitted through the seed in which the organism has been known to remain alive and virulent for five years.

As a control measure, the use of clean seed from wilt-free fields gives the most hope of success at present. The use of bean straw as fertilizer or feed should be avoided. Experiments on seed treatment and soil infection by *Bact. flaccumfaciens* are under way.

A technical dsecription of the causal organism and a detailed account of its cultural characteristics are given.

Some comparisons of *Bact. flaccumfaciens* and *Bact. phaseoli* are given, and a table of cultural tests for differentiating the two organisms is appended.

BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

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EXPLANATION OF PLATES

PLATE I.

Pods and seeds infected with *Bact. flaccumfaciens*
Photo. by James F. Brewer

A. Badly infected seeds in pod of Navy pea bean which showed no sign of infection externally (compare B). Bacterial layer underneath the seed coat. Very infectious colonies plated from X. Coll. Michigan, Sept., 1922. About natural size.

B. Exterior of pod shown in A.

C. External infection on mature pods of Early Wonder. Olive greenish discoloration along suture. One of these might be easily overlooked. All contained infected seed. Coll. Michigan, Aug. 24, 1923. Natural size.

PLATE II.

Bean plants infected with *Bact. flaccumfaciens*
Photo. by James F. Brewer

A. Lazy Wife pole bean 2 months after prick inoculation below first pair of leaves. Whole top of main stem defoliated and dead. All leaves on lateral branch discolored and wilted. Pod contained diseased seed. ¼ nat. size.

B. Navy bean 9 days after prick inoculation below first pair of leaves. ¼ nat. size.

C. *Bact. flaccumfaciens* on 7 varieties of beans 4 weeks after inoculation by pricking. 1/6 ca. nat. size.

D. Checks for preceding.

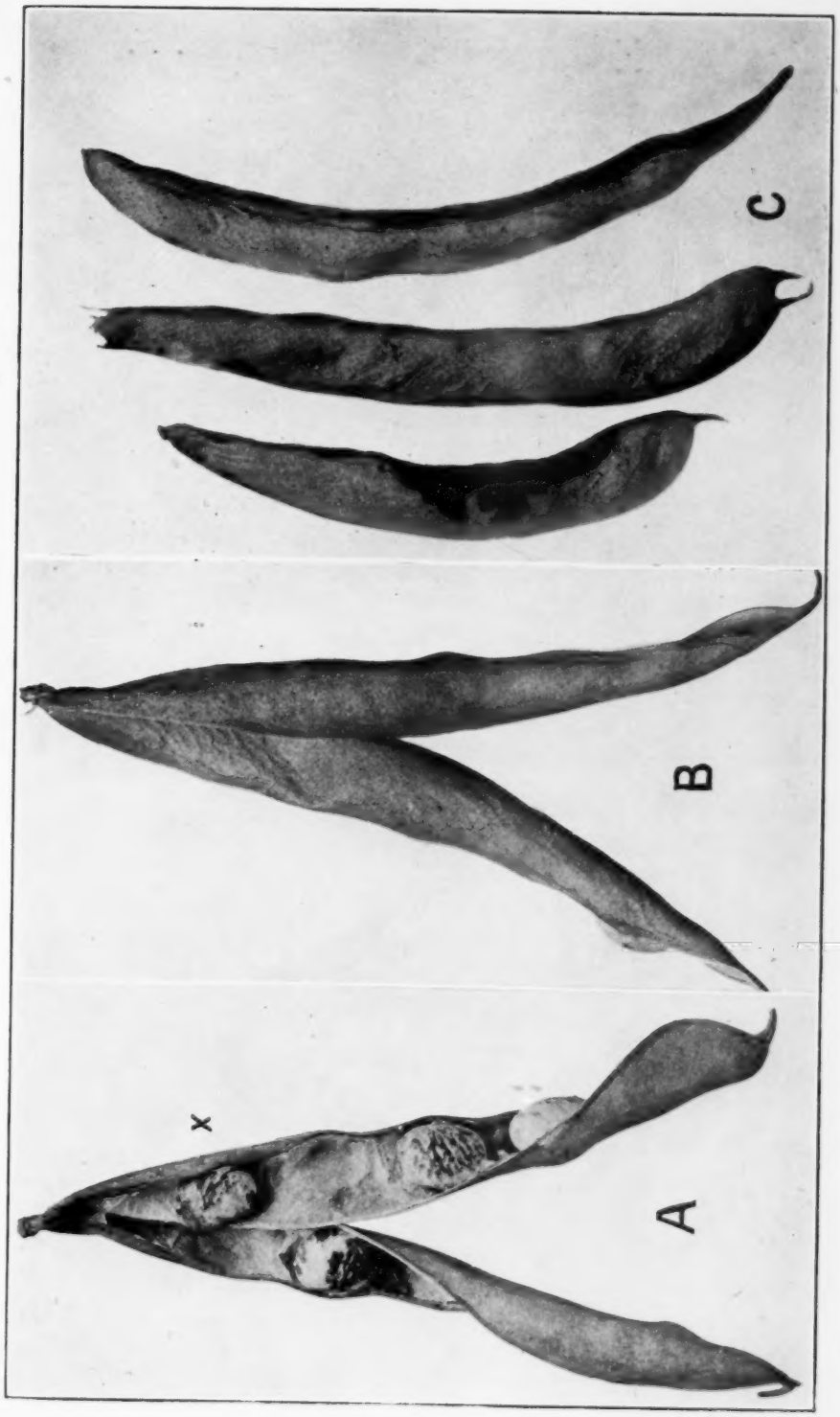
E. Wilt in Navy bean plants grown from diseased seed planted in sterilized soil in the greenhouse 5 weeks old. ¼ ca. nat. size.

PLATE III.

Pods and seed infected by inoculation with *Bact. flaccumfaciens*
Photo. by James F. Brewer

A. Pods of Lazy Wife pole bean 14 and 15 days after prick inoculation showing greater susceptibility of the seed. Natural size.

B. Pods of Lazy Wife pole bean 4 weeks after prick inoculations in the pedicels. Pods withering and yellowish green. Seed infected. Natural size.



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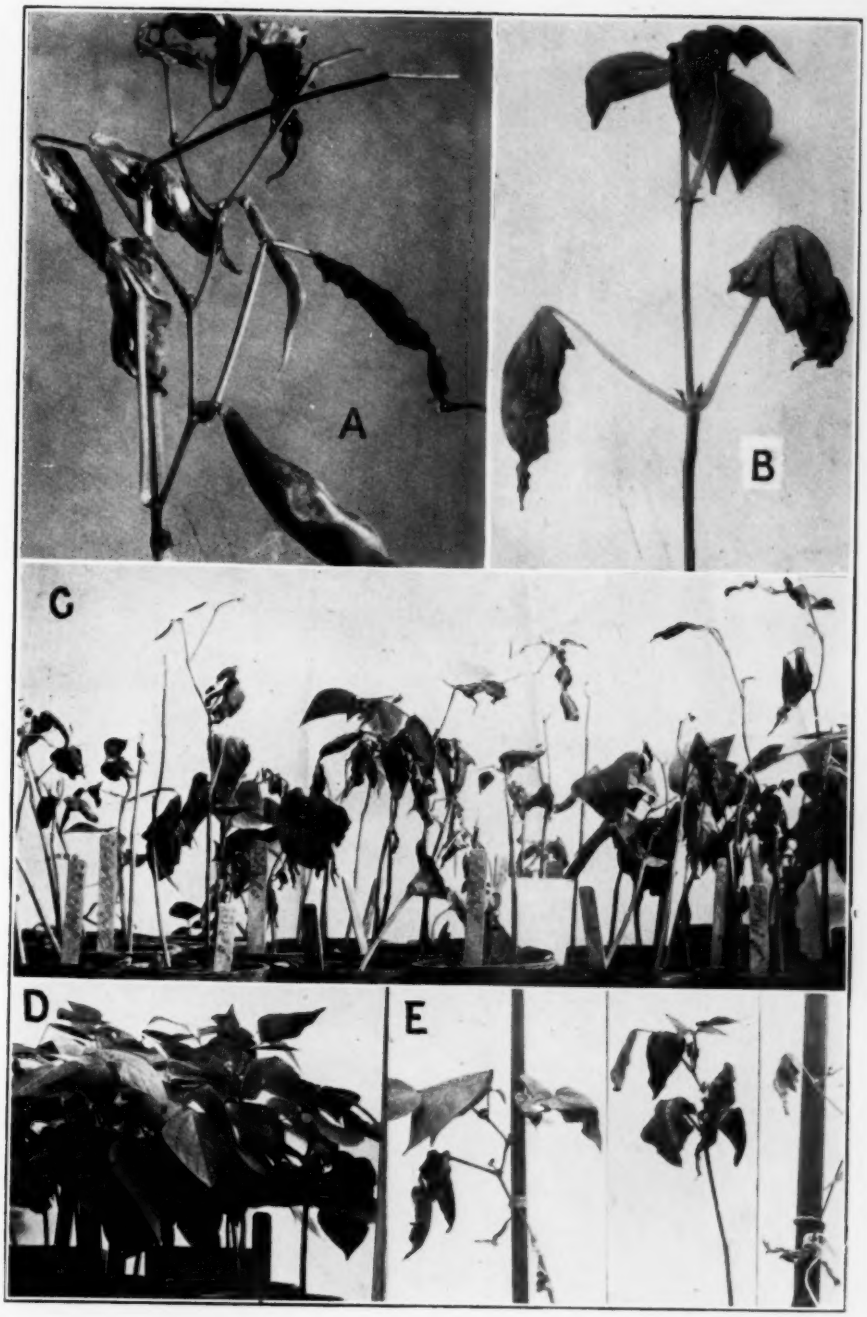

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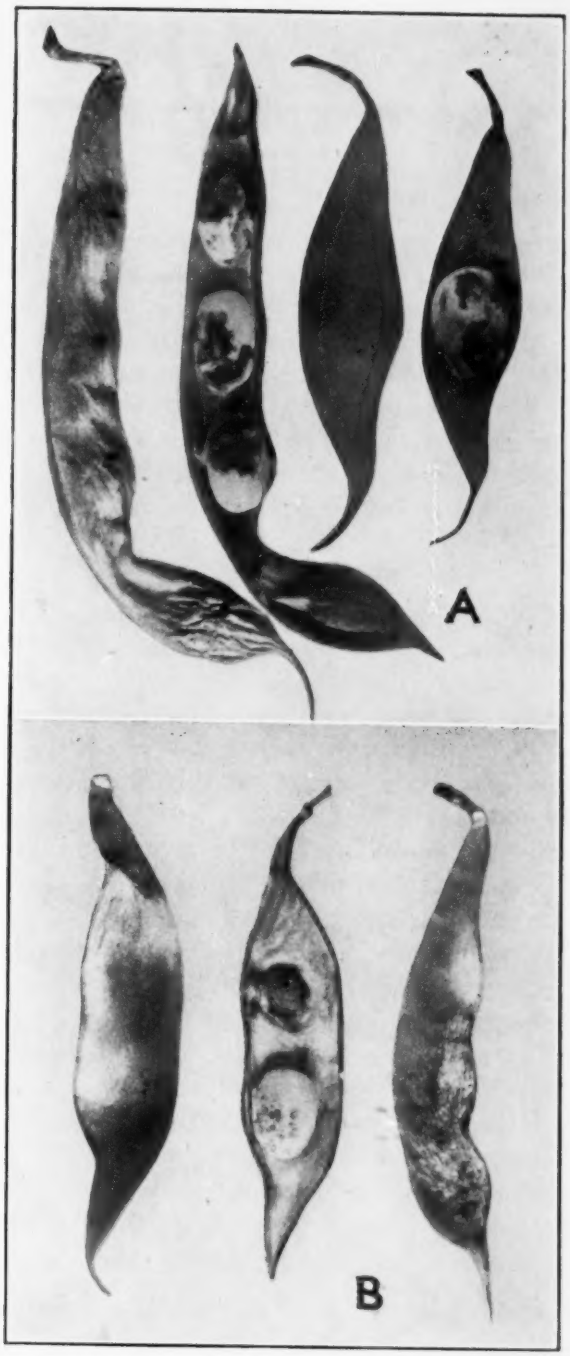

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THE "BLACK DOT" DISEASE OF POTATO[1]

B. T. DICKSON

The increasing importance of the potato crop to human welfare needs no stressing, and one has constantly in mind the numerous diseases to which that crop is heir. This paper deals with a disease which is quite cosmopolitan in distribution and yet comparatively little known. Such a statement justifies the inference that it cannot be of prime importance at the moment; nevertheless, information regarding it should be available. The study has been carried on since the late summer of 1921, and further work is still necessary to determine accurately its economic significance. It is variously known as dartrose, anthracnose, foot rot, and black-dot disease.

HISTORICAL

In the summer and early autumn of 1921 at, and in the vicinity of, Macdonald College, the author observed a peculiar premature dying down of the tops of potato plants, and investigation revealed the fact that the condition of the plants was due to attacks by a fungus belonging in the *Colletotrichum-Vermicularia-Volutella* group. This was reported (13) under the name "black dot" disease, which is an excellent descriptive name given by McAlpine (20) to a similar affection of potatoes in Australia; and, since what appeared to be the same disease had been ascribed by Ducomet (16) to *Vermicularia varians*, the present author also ascribed it temporarily to that organism. However, certain growth characters of the organism rendered it necessary to make a more detailed study, the results of which appear herein.

A survey of the literature available gives the following references to the same or closely related diseases and organisms.

In 1825, Link (19) described *Exosporium maculans* as occurring on potato stems in France and Belgium. Fries, in his index to Volume III of the Systema Mycologicum (1829), gives *E. maculans* Lk. as a variety of *Vermicularia dematium* Fr. which also occurs on potato stems. While spore sizes are approximately the same, those of *V. dematium* are falcate, and those of *E. maculans* straight or but slightly curved. On this basis

[1] Read before the Botanical Section of the British Association for the Advancement of Learning at the Toronto Meeting, August, 1924.

Desmazieres (12) renamed it *Vermicularia maculans,* which was accepted by Fries in his Summa vegetabilium Scandinaviae . . . of 1849.

Berkeley & Broome (2), in 1850, reported *Vermicularia atramentaria* on decaying potato stems, forming inky-black patches and distinguished at once by its straight spores. Concerning *V. atramentaria,* the statement appears in the Sylloge Fungorum III, page 228: "*Verm. maculans* (Lk.) Fr. Summa V. S. p. 420, Kickx Fl. Fl. I, p. 405, valde affinis est, nisi eadem ac. *Verm. atramentaria* B. et Br."

Caspary (7) published an account of a similar organism found on potato, which he called *Steirochaete solani.* The genus *Steirochaete* A. Br. et Casp. is recognized in the Sylloge Fungorum, page 592, as: "verisimiliter totum genus cum *Colletotrichum* confluit" (see also Eng. u. Prantl. I, 403); and concerning *S. solani,* Caspary is quoted: "An eadem ac *Vermicularia maculans?*"

Between 1841 and 1859, Westendorp (28) noted *Vermicularia herbarum* on decaying stem of potato and other plants. In the Sylloge Fungorum III, page 226, the spore size is given as 3–4 μ by 20–22 μ, and the following statement occurs: "Longuis distat a *Verm. atramentaria* B. et Br. (? = *Verm. maculante* (Lk.) Desm.) ob perithecia majora ab laxe sparsa."

Hallier (17), in 1875, considered that a Krauselkrankheit of potatoes was due to an organism developing black sclerotial bodies clothed with black setae, which he named *Rhizoctonia tabifica.* This particular organism Pethybridge (23) showed to be the sclerotial stage of a *Colletotrichum,* which he therefore re-named *Colletotrichum tabificum* (Hall. pro parte).

In 1881 Saccardo and Roumeguère (25) recorded *V. orthospora* with spores 4 x 22 μ occurring on potato stems, and about the same time *V. eupyrena* with spores 4–4.5 by 20–26 μ occurring in "caule denique decorticate emortuo *Solani tuberosa*" was noted by Saccardo (24).

In 1886 Brunaud (6) described from the environs of Saintes, France, *Sclerotium solani* characterized by a black interior on dry potato stalks in winter. No spores were found, but it may be the same organism with which we are concerned in a slightly modified condition.

In 1894 Halsted (18), while working with potatoes, observed in connection with a stem blight a fungus which he considered to be *V. atramentaria* B. et Br., and regarding which he reported: "the amount it has to do with causing the destruction of the crop is an open question."

Recent attention was first drawn to the disease by Ducomet (16), who described the organism from France as *Vermicularia varians,* such species name being suggested, no doubt, by the final statement of his description: "Espèce variable, évoluant vers le *Phoma* ou inversement vers les *Colletotrichum* et *Gloeosporium.*" McAlpine (20) noted its occurrence in Aus-

tralia, pointing out that he could not find pycnidia in any of his specimens. In the same year, Manns (21) studied dry rot of the potato due to *Fusarium oxysporum* Schlecht, and observed: "it is common to find associated with the *Fusarium*, and likewise penetrating the tuber sometimes to a depth of one-fourth to one-half inch, a fungus of the genus *Vermicularia*."

Doidge (15) reported from South Africa what is apparently the same disease as that described by Ducomet.

In 1915, O'Gara (22) published a full description of *Colletotrichum solanicolum* sp. nov. which he found in many potato fields in Salt Lake Valley, Utah. The organism confined its attacks mostly to underground parts, invading the cortex. The spore size given is 3.5–5 by 17–22 μ, and he notes that the organism is related to *Vermicularia*.

Taubenhaus (27), in the following year, dealt with silver scurf of the potato, and in the course of his studies decided that *Phellomyces sclerotiophorus* Frank is the same as *Vermicularia atramentaria* B. & Br., and the same as *Colletotrichum solanicolum* O'Gara. On page 355 he also noted that Dr. Manns pronounced his *Vermicularia* identical with *Colletotrichum atramentarium* (B. & Br.) Taub.

In 1916, Bewley (3) gave a description of a tomato disease which was attributed to *Sclerotium setosum* Bewley and Shearn. In correspondence Dr. Pethybridge informed the writer that he had submitted a culture of *C. tabificum* to Dr. Bewley, who had agreed that *S. setosum* was the same as *C. tabificum* (4).[2]

In 1923, Averna-Sacca (1) reported a destructive anthracnose of potato from Sao Paulo, Brazil. Affected plants could be pulled without effort, and under the cortex of subterranean parts *C. solanicolum* O'Gara occurred. The spore size given by Averna-Sacca differs somewhat from O'Gara's in width.

Brittlebank (5), in a discussion of tomato diseases, states that "black dot" root disease has caused considerable loss to tomato growers for the last fifteen years in Victoria, Australia. Recently Chaudhuri (9)[2] published an account of *Colletotrichum biologicum* also causing anthracnose of potato in Germany.

GEOGRAPHICAL DISTRIBUTION

From the above historical account it would appear that the same or closely similar diseases are cosmopolitan in occurrence. Localities from which reports are available to the writer include Quebec, New Brunswick, and Prince Edward Island in Canada (Dickson); New Jersey (Halsted,

[2] Received since the B. A. meeting.

18), Ohio (Manns, 21), Utah (O'Gara, 22), Florida in 1905, Oregon in 1916 (Dr. W. W. Gilbert in correspondence with the author), Pennsylvania in 1921–24 (W. A. McCubbin and Dr. Freeman Weiss in correspondence), Delaware in 1916 (Taubenhaus) and West Virginia in 1922 (Dr. N. J. Giddings, in correspondence) in the United States of America; England (Pethybridge); Ireland (Pethybridge, 23); Australia (McAlpine, 20); South Africa (Doidge, 15); France (Ducomet, 16); Brazil (Averna-Sacca, 1); Germany (Berkeley & Broome, 2, and Chaudhuri, 9); Italy (2 and 24); and Belgium (2 and 28).

SYMPTOMS

General. The first symptom is a slight yellowing of the foliage, beginning at the tips and gradually involving entire leaflets. At the same time a rolling may be noticed similar to that occurring in such stem infections as those caused by *Rhizoctonia solani*, *Fusaruim oxysporum*, and *B. atrosepticus*. This symptom has been well described by Crepin (10), who calls it "enroulement flasque" (flaccid rolling) and differentiates it from the condition in "leaf-roll" or "Leptonecrosis" of Quanjer. It may appear comparatively early in the season if infection is heavy and early, and if the weather becomes dry, but usually it is a midsummer to autumn symptom. In ordinary cases the foliage gradually yellows, droops, and dries up, so that affected plants present the appearance of dried mature tops some three weeks earlier than unaffected plants in the same plot. In dry periods, the shrivelling and browning of the foliage and stems occur much more rapidly, as was the case with certain plants in 1921. Crepin notes that where desiccation is relatively slow, axillary buds become swollen, and in certain varieties (Hollande du Gatinais, Violette du Forez, etc.) aerial tubers develop, sometimes abundantly.

Early infection, especially if accompanied by adverse environmental conditions, causes a definite dwarfing of parts of the plant developing under those conditions.

Underground Parts

Stems. The stem is commonly affected from just about ground level down to the parent tuber, the actual zone being determined by whether infection occurred from sclerotia on the parent tuber or from the soil or debris. The cortical tissues are invaded and broken down so that it is easy to scale them from the dry stem, as shown in Pl. IV, fig. 3. The mycelium fills the vascular tissues and appears on the inner circumference of the vascular cylinder. When the tissues are drying, it is not uncommon to observe, after peeling the remnant of the cortex, a delicate but definite

amethyst coloration, which is very marked on the inside of the vascular cylinder. Pethybridge (23) draws particular attention to this apparently usual coloration in material in England and Ireland.

When desiccation commences, the sclerotial bodies develop in varying numbers and with or without setae. They may not only line the vascular cylinder on both inner and outer periphery but often occur in considerable numbers on the inner surface of parts of the cortical cylinder, which by this time is loosened and easily removable.

Stolons. Similar conditions hold for stolons as described above for stems, but it is noticeable that relatively more lesions completely involving the tissues occur in stolons than stems. This is of importance in affecting yield, since stolons may be attacked at any stage of tuber development. Usually the lesion severing connection along the stolon is found from ½ to 1½ inches from the young tuber. When the tuber is dug thus incompletely matured, it can be seen to carry at its stem end a varying length of dried stolon which may or may not show sclerotia. The author has examined many potatoes from widely scattered sources, and in almost every case the adherence of a part of the stolon to a tuber is an indication of the presence of this disease.

Tubers. The tubers are affected in their development by the cutting off of food as a result of stolon lesions, and, since this may occur at any stage in growth, it is possible to find tubers just commencing to swell, through all grades to large mature potatoes separated from the parent system. If very small, the tubers soon rot or shrivel up, but larger potatoes may remain until harvest and show no other symptoms than small size and the attached fragments of stolons. On the other hand, such tubers may be covered on the upper surface, all over, or in patches, with sclerotia. This may also happen with a tuber of which the stolon is unaffected. The aggregation of sclerotia over the surface varies from rather scanty to close; for example, on one tuber the estimate was approximately 300 in a square inch.

On freshly dug white potatoes, the writer has seldom observed the greyish areas, somewhat resembling silver scurf, mentioned by Ducomet (16) and Crepin (10), and because of which the former named the disease "Dartrose." Ducomet states that this condition is more easily seen after the potatoes have been stored some time, and this the writer can corroborate. Pigmented varieties have not yet been studied, but both the above-mentioned French authors agree that on purple and red varieties the rather rough decolorized plaques are easily seen.

Roots. The roots are attacked in all stages of development, and they also are decorticated. When the roots are pulled, it is usual to observe the

broken naked vascular cylinder with attached fragments of desiccated cortical tissues carrying sclerotia. The root lesions account for the ease with which one may pull up the stems of affected plants, since the roots break about two inches from the stem.

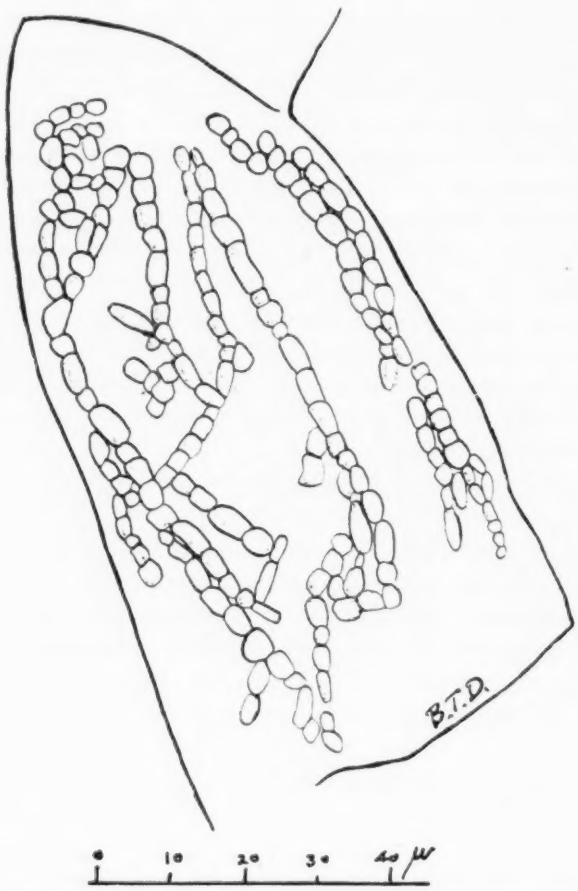

FIG. 1. Mycelium in trichome cell of completely parasitized potato plant. Camera lucida drawing.

PATHOLOGICAL HISTOLOGY

The author's studies of the pathological histology have so far not been systematic, but in general they confirm the careful work of Ducomet (16). In one notable case, a greenhouse plant artificially inoculated indicated a complete parasitism. The mycelium apparently grew rapidly up the vascular cylinder of the stems into the leaves, and invaded even the trichomes (Fig. 1).

ETIOLOGY

Ducomet (16) affirmed: "Le parasitisme de notre éspèce n'est pas douteux." He pointed out (p. 42) that when Delacroix was studying his *Bacillus solanincola* in 1901 he also found a *Fusarium* which appeared to correspond with *F. lycopersici* Sacc., and a second fungus which he thought ought to be called a species of *Vermicularia*. Manns (21) found a *Vermicularia* present to the extent of 10.3 per cent among his isolations when working with *Fusarium oxysporum* Schlecht. He states (p. 316): "When used alone it brings about no disease symptoms. If it assists any in bringing about the disease, its work is that of a semi-parasite which follows the openings made by the *Fusarium*. It is almost invariably present on the underground stems and roots of sick plants in the field, and upon tubers which are carrying internally the *Fusarium* fungus, showing plainly by means of the hand lens at the point of the stem connection. The writer has many times taken it from internal parts of the roots and underground stems of potato plants which were showing only the first symptoms of *Fusarium* blight."

Crepin (10) finds that it is common on plants killed by attacks of late blight in late August. In Bretagne and at Grignon: "il est vraisemblablement saprophyte et il nous apparait comme étant très répandu."

In the original isolations of 1921, the author also found in many plates that a *Fusarium* (probably *F. oxysporum*) appeared in the cultures, and, because of its rapid growth, that organism overran the black-dot organism. The latter invariably appeared, however, and the fact remains that in approximately half the plates out of some eighty it came up pure or contaminated with bacteria, and in three cases contaminated also with *Rhizoctonia*.

Dr. G. H. Pethybridge was good enough to send, early in 1924, some material collected in Lancashire, England, in December, 1923. Part of this was used for isolation, and a *Fusarium* appeared in ten of the cultures out of twelve, the two others giving pure plates of the black dot organism.

Undoubtedly, therefore, a *Fusarium* is very frequently associated with our present fungus, but so far the writer's work has led him to believe that the *Fusarium* is secondary or associated with the *Colletotrichum*.

Experiment 1

During the fall of 1921 careful collections were made, from affected Green Mountain plants, of tubers carrying numerous sclerotia. They were washed in mercuric bichloride solution (1 part in 1,000), for 30 minutes, and eleven hills were planted in the spring of 1922 in the experimental

plots of the Department of Botany at Macdonald College. The plants grew poorly; they were stunted and weak, and showed all symptoms of dartrose. By September 18, all the tops were dead, while adjoining rows were full of green tops. The largest tuber obtained was 2 inches long, and the majority were about one inch. Every stem exhibited abundant sclerotia, and many showed the amethystine coloration described by Pethybridge (23). There were ample evidences of stolon infection and the subsequent development of new stolons which in turn were attacked. The tubers were plentifully besprinkled with sclerotia. Isolations from the stem gave cultures of the organism often contaminated by bacteria but rarely by *Fusarium*.

Experiment 2

At the same time a plot of 64 Green Mountain potatoes, with eight hills per row, was planted. On July 4th, when the plants were about 3 inches high, the following inoculations were made:

Row 2, with the organism isolated by the author.

Row 4, with cultures of *C. atramentarium* (by kindness of Dr. Detmers).

Row 6, with cultures of a *Colletotrichum* from West Virginia (sent by Dr. Giddings).

The inoculations were made by carefully removing the soil to a depth of about three inches and placing on the exposed stem an agar culture about one-quarter inch in diameter. Every effort was made to avoid injury to the stem and roots. The results observed on September 20 were as follows:

Row 2, seven plants dead, all stems with sclerotia.

Row 4, six plants dead, all stems with sclerotia.

Row 6, four plants dead, all stems with sclerotia.

Rows 1, 3, 5, 7 and 8, plants all green; free from disease.

Isolations from inoculated plants again gave cultures of the black-dot organism contaminated by bacteria, but only in three plates out of eighteen by *Fusarium*. The similarity of the organisms used will be discussed below.

Experiment 3

In the field it is always possible that *Fusarium* spp. are present in the soil; in order to obviate this, a set of greenhouse tests were started on February 21, 1924. Green Mountain potatoes were treated by the hot formaldehyde method and planted in pots. Both pots and the soil therein had been thoroughly autoclaved. On March 15, when the plants were about 3 inches high, they were inoculated. An examination of the plants on June 1 gave the results shown in table 1.

TABLE 1.—*Results of inoculating potato plants growing in pots of sterilized soil*

No. of plants inoculated	Inoculum	Source	Results
2 (A)[a]	Quebec organism	Dickson	Plants yellowed, wilting unless well watered; stem, stolons, roots, and tubers with sclerotia
2 (B)	do	do	do
2 (A)	Saltation	Dickson (14)	Plants showed symptoms but very few sclerotia
2 (B)	do	do	One plant do
2 (A)	*C. tabificum*	Isolated from material sent by Dr. Pethybridge	Two plants typical as above
2 (B)	do	do	Two do
2 (A)	*V. varians*	Isolation by Cavadas	Two do
2 (B)	do	do	One do
2 (A)	*C. atramentarium*	Isolation by Dr. Detmers	One do
2 (B)	do	do	Two do
2 (A)	*Collecotrichum* sp.	Isolation by Dr. Giddings	Two do
2 (B)	do	do	One, slight infection; no obvious symptoms

[a] Series (A): Inoculations with injury by scalpel to stem.
" (B): Without apparent injury.

In the above experiment every effort was made to avoid contamination by other fungi, and isolations from the soil at the end of the test gave no indication of the presence of *Fusarium* spp. The similarity in results obtained with the different cultures will be dealt with later.

Field tests were made during the season of 1924 in a plot which had not been planted with potatoes for at least twelve years. The ground was a heavy clay loam, which was very wet during the spring and very dry during June. Disease-free seed was planted on June 9; and on July 3 and 4, six hundred and thirty hills were inoculated as indicated in figure 2. Each plant was exposed just below ground level with as much avoidance of injury as possible, and a small mass of potato dextrose agar culture or

dry stem material was applied to the stems, which were at once recovered.

Examination was made on Sept. 20, 1924, when it was comparatively easy to detect prematurely dead tops. The crosses in the diagram (Fig. 2) indicate the affected plants. It was noteworthy that in every series an amethystine coloration was present on most of the diseased stems. In the case of the saltation there were few sclerotia, but they were definitely pres-

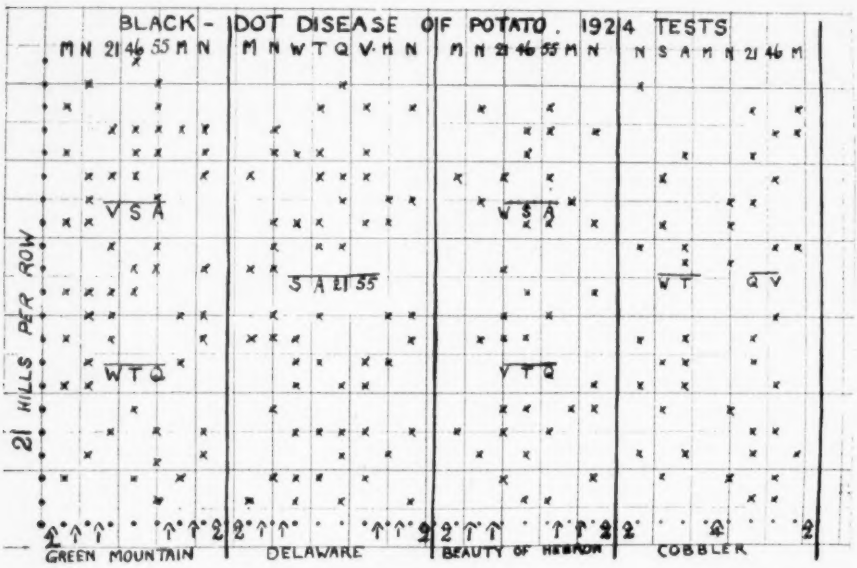

FIG. 2. Diagram of the 1924 experimental plots in which four varieties of potato were inoculated with eleven different black-dot cultures. Crosses (x) indicate infected plants. 1, 2, 4 = number of check rows between inoculated rows; M = fragments of stems with sclerotia collected during 1922 and kept dry in a cool place; N = fragments of stem with sclerotia collected during 1923 and kept dry in a cool place; S = saltation; V = subculture by author from original by Cavadas through Dr. Foex and Dr. Pethybridge; Q = organism isolated in Quebec; T = isolation by author from Lancashire, Eng., material sent by Dr. Pethybridge; W = subculture from original by Dr. Giddings from West Virginia; A = subculture from original by Dr. Detmers from Ohio; 21 = subculture from original by Dr. Weiss (from wilted plant); 46 = subculture from original by Dr. Weiss (stem-end rot of tuber); 55 = subculture from original by Dr. Weiss (from wilted plant).

ent. It would appear from this one season's inoculations that Green Mountain and Delaware are somewhat more susceptible than Beauty of Hebron and Cobbler. Except with the saltation, it was impossible to distinguish any one series from another, either by the effects on the stem, by coloration produced, by sclerotia, or by spores. From this it is justifiable to infer that the various inocula used from such widely distributed sources are of the same cosmopolitan organism. This confirms cultural studies on these isolations carried on by Scott (26).

Pethybridge (23) inoculated healthy potato stalks and tubers through wounds, but, beyond a strictly limited growth in the wound, no development occurred; and no trace of any kind of rot was set up. He concluded: "there may possibly be special conditions under which it behaves as a parasite or at least as a feeble parasite."

Cavadas (8) carried out some tests in the greenhouses of the Station de Pathologie Végétale of Paris. In two pots the soil was inoculated with conidia, in two others with sclerotia; two tubers with conidia, two tubers with sclerotia; two plants on the wounded stem at ground level when 8–12 cm. high; two with conidia and sclerotia without wounding when the plants were 8–12 cm. high; two plants were left as checks.

Characteristic symptoms were evident in the plants from inoculated soil and tubers. With the wounded plants the symptoms were less definite, and it appeared that the plants reacted against the parasite to limit its action on the inoculated part. The plants inoculated (disseminé) without wounding remained healthy. Cavadas concludes that *Vermicularia varians* Duc. becomes a true parasite and can give rise to serious losses only when it attacks the plant in early stages of growth. In other cases it is inoffensive, but it can help in the destruction of the plant if some factor diminishes resistance.

With these conclusions the author can generally agree, as a result of the experimental work so far completed. Nevertheless, without further and wider observation it is not possible to determine the economic importance of the organism, although the author believes it to be more important than is generally believed.

MORPHOLOGY OF THE ORGANISM

In the course of three years' culturing, the organism has been grown on various media, among which the best were potato-dextrose, Czapek's, oatmeal, and cornmeal agars. Since potato-dextrose was used most, it will be the one considered in the following description.

From single spores the first indication is a white, silky, superficial mycelium, spreading at the rate of about 8 mm. a day at 21° C. The hyaline cells are 10–35 μ long by 3.5–5 μ in diameter. In about four days an amethystine tint, most pronounced in the centre of the culture, begins to appear in the submerged mycelium. In a few plates a yellow coloration may be evident. Dr. Detmers, in correspondence of March 30, 1923, concerning *C. atramentarium*, notes that in media of high sugar content "the conidial masses show a characteristic yellow, almost apricot yellow color." Our media contained one per cent of sugar, and the yellow or amethyst

coloration was always a sign of superabundant spore formation. In about six days sclerotia begin to appear in concentric rings, and the superficial mycelium becomes less apparent until the whole plate is covered with sclerotia. On Czapek's and cornmeal agars the sclerotia are more widely placed than on dextrose agar, on which medium they become so numerous that they tend to coalesce, and often eventually form an almost solid black cake. Conidia germinating and growing on the surface of this again give a coating of white mycelium which is usually rather patchy in development.

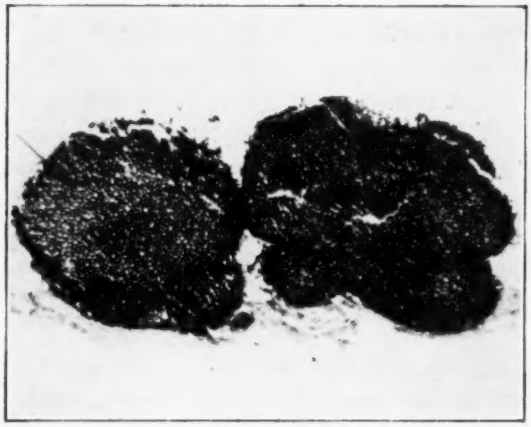

FIG. 3. Photomicrograph of cross-sections of sclerotia of Quebec organism. No evidence of pycnidia. Cultured on potato-dextrose agar. ×75.

The black sclerotia, with or without setae, vary in size from 100 microns to ½ mm. (Fig. 4). When young they contain oil, and when mature are made up of a dark, thin-walled pseudoparenchymatous tissue (Fig. 3), the cells of which are 4–9 μ by 10–14 μ in size. No pycnidia have ever been observed in our cultures.

The setae develop as usual with *Colletotrichum*, and vary in length from 80 to 350 μ, tapering from a somewhat swollen basal cell of 5–7 μ diameter, hyaline at the tip, but darkening with age and showing two to four septa. As many as 70 setae have been counted on a single sclerotium. They do not always appear in culture, especially if the organism has been subcultured for a considerable period. In nature they may not always be present, but on the other hand they can often be seen with the naked eye, especially on the outer periphery of the vascular cylinder.

The conidiophores develop free or in palisade layers and are subhyaline, 10–30 μ long, cylindrical, tapering to slightly clavate, sometimes curved, occasionally branched and from 0 to 3-septate.

Conidia are often developed in tremendous numbers, especially in the case of the saltation (14). The writer has counted as many as 174 spores, lying like logs in a timber raft, which apparently had developed from 15 conidiophores. They are pink in mass (except saltation, in which they are yellow), hyaline individually, continuous, sometimes slightly curved, somewhat attenuated at the basal end and round at the apical end, 3–7.5 μ by 17.5–22 μ, and 1 to 3-guttulate.

The germ tube usually develops at the apical end or just to one side, and appressoria are common in hanging-drop cultures. At times, what appears to be a septum is seen in the conidium just at germination, and a noticeable feature is a swelling of many of the spores to almost twice their original diameter, probably due to hydrolysis of some constituent of the spore contents with the consequent absorption of water.

Since this paper was read, the author has received from Dr. Chaudhuri a culture of his recently described *C. biologicum* (9). Dr. Chaudhuri differentiates his organism from that of Dr. Pethybridge by the absence of amethystine fluorescence. Remarkably enough, our first subculture made on oatmeal agar developed a beautiful amethyst coloration, and this has since persisted in subcultures. Chaudhuri also points out that the sclerotia are larger than those of *C. solanicolum* and the conidiophores longer. The present author has found that sclerotial size is somewhat unreliable, inasmuch as considerable variation may occur; and conidiophores as long as 30 μ have been found in subcultures from Dr. Detmer's isolation. He also finds two sizes of conidia, *viz.*, 5 x 22 μ and 5 x 14 μ. So far, the average size of conidia obtained in our subcultures is 3.75 x 17.5–20.6 μ, and we have not observed any definite differentiation into the two groups, although some spores are but 10–15 μ long. Dr. Chaudhuri remarks on the resemblances between *C. solanicolum, C. atramentarium, C. tabificum,* and *C. biologicum,* and as a result of preliminary studies on *C. biologicum* it appears to the present author to be so closely similar as to justify its being regarded as the same.

TAXONOMY

Link (19), in 1825, described *Exosporium maculans* as occurring in winter on dried stems of herbaceous plants; and Fries, in the index (p. 89) to Volume III of the Systema Mycologicum, noted it as a variety of *Vermicularia dematium* Fr. In 1828, Desmazières (12) named it *Vermicularia maculans,* for which the following description appears in Sylloge Fungorum **3**: 228:

"*Vermicularia maculans* (Link) Desm. Exs. n. 339, *Exosporium maculans* Link Sp. plant. Fung. II p. 123.—Peritheciis innatis, minutis, aggregatis, dein confluentibus et matricem nigrificantibus, rotundatis, applanatis, setulis concoloribus, sparsis, fasciculatis, apicem versus attenuatis, divaricatis obsitis, denique apice hiantibus; sporulis (paucis) elongatis vel oblongis, rectis, subobtusis.
Hab. in caulibus plantarum herbacearum, praecique *Solani tuberosi* et Utricae urentis in Gallia et Belgio."

Berkeley & Broome's (2) *Vermicularia atramentaria* appeared in 1850, and the technical account in Saccardo's Sylloge Fungorum, p. 227, is given herewith:

"*Vermicularia atramentaria* B. et Br. Ann. N. H. n. 430.—Effusa, gregaria, atra, maculiformis; peritheciis minutis superne setulis longis rectis, basi vero fibris repentibus, subepidermicis cinctis; sporulis minutis, cylindraceis, brevibus, utrinque nucleatis, (plasmate utrinque retracto, Berk.).
Hab. in caulibus *Solani tuberosi* in Britannia, Italia, Gallia, Belgio, Germania.—*Verm. maculans* (Link) Er. Summa V. S., p. 420, Kickx Fl. I. p. 405, Valde affinis est, nisi eadem ac *Verm. atramentaria* B. et Br."

The statement regarding the close similarity, if not identity, of those two organisms is noteworthy. By the kindness of Dr. A. W. Hill, Director of Royal Botanic Gardens at Kew, England, the author has been able to compare them, and finds that the only difference discernible in the exsiccati specimens is that of spore shape; but this appears to be sufficiently definite to separate them. Those of *V. atramentaria* (*C. atramentarium*) are straight cylindrical, rounded at the apex, and slightly tapering at the basal end, while those of *V. maculans* (*C. maculans*) are straight to slightly curved, appearing from a lateral view short glume-shaped in outline.

Taubenhaus (27) studied the specimens of *Vermicularia atramentaria* of the New York Botanical Garden, and, concluding that the fungus fits in better with *Colletotrichum*, named it *C. atramentarium*. His own cultures were submitted to Dr. O'Gara, who pronounced it the same as the latter's *C. solanicolum*. Taubenhaus was studying silver scurf (*Spondylocladium atrovirens*) of the white potato, and held the view that the sclerotial bodies he found were the same as *Phellomyces sclerotiophorus* Frank, which he therefore decided to be an invalid type, since his sclerotial bodies gave rise to the *Colletotrichum* cultures.

In material obtained from New Brunswick the present author was fortunate enough to culture from the same tubers both the *Colletotrichum* and *Spondylocladium*—the latter from sclerotial bodies which were undoubtedly stromata of *Spondylocladium atrovirens* Harz. It is thus apparent that both sclerotial bodies may be present on the same tubers, and

we therefore cannot agree with Taubenhaus in his conclusion regarding the invalidity of *Phellomyces sclerotiophorus* Frank, although this does not contradict his view that the *Colletotrichum* is the same as *V. atramentaria* B. & Br.

Crepin (11) notes that "Dartrose et gale argentée se presentent sous un aspect assez analogue et il faut quelque attention pour les distinguer."

Since the B.A. meeting the author has had the opportunity of comparing exsiccati specimens from Kew of *V. herbarum* and *V. orthospora* (= *C. orthosporum*). He was unable to find setose sclerotia, conidiophores and

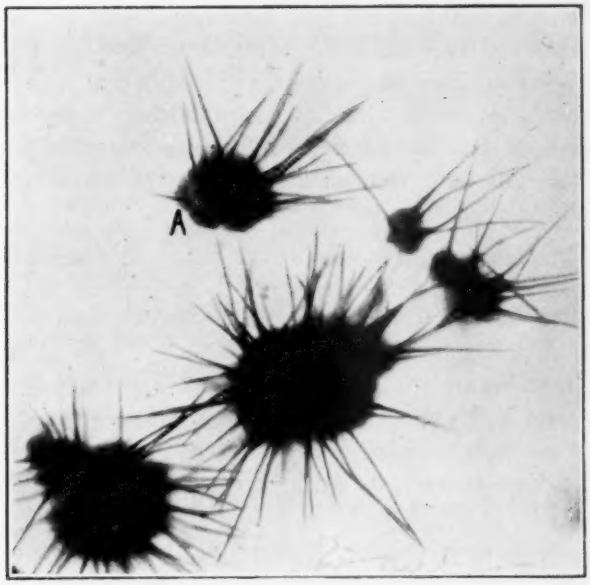

FIG. 4. Sclerotia with setae, Quebec organism. Tips of spores visible at A. ×100.

conidia of *V. herbarum*, and therefore unable to decide regarding that organism. *V. orthospora* was closely similar in all respects to our organism, except that all the sports were typically larger. It is obviously closely related, but, judged by spore size, is not identical.

Summing up such taxonomic studies as have been made, it may be stated:

1. That the organism causing black-dot disease, anthracnose, or foot rot of potato is properly known as *Colletotrichum atramentarium* (B. & Br.) Taub.

2. That the following organisms are the same and therefore their names synonymous: *C. tabificum* (Hallier pro parte) Pethy., *Rhizoctonia tabifica*

Hall. (17), *C. solanicolum* O'Gara, *Vermicularia varians* Duc., *Sclerotium setosum* Bewley and Shearn, and probably *C. biologicum* Chaud.

3. That *C. orthosporum* (Sacc. et Roum.) nov. comb. is very closely related.

CONTROL

No definite experiments relating to control have been undertaken, but from general observation certain facts are plain. The disease often occurs in poorly drained sections of otherwise healthy fields of potatoes. The avoidance of such areas is therefore advisable. The sclerotia of the organism may remain in plant debris, and perhaps in soil, for more than one year; and, if the soil becomes badly contaminated, rotation must be practised. Since sclerotia develop on tubers and adherent stolon pieces, seed-tuber treatment, preferably with hot formaldehyde, is necessary. Insufficient work has been done to warrant conclusions as to varietal susceptibility, but Green Mountain and Delaware appear to be more susceptible than Beauty of Hebron and Cobbler.

SUMMARY

1. Black-dot disease, dartrose, anthracnose, or foot-rot of potato is widely distributed.

2. The symptoms are similar to those of other diseases due to organisms attacking cortical and vascular tissues at and below ground level, but not so likely to be acute because of the rather weak parasitism of *C. atramentarium*. On tubers there is frequently considerable simulation of silver scurf.

3. *C. atramentarium* is a weak parasite, attacking mainly debilitated plants or young plants which succumb in early maturity.

4. Comparative studies show that organisms from England, France, Germany, United States of America and Canada are the same.

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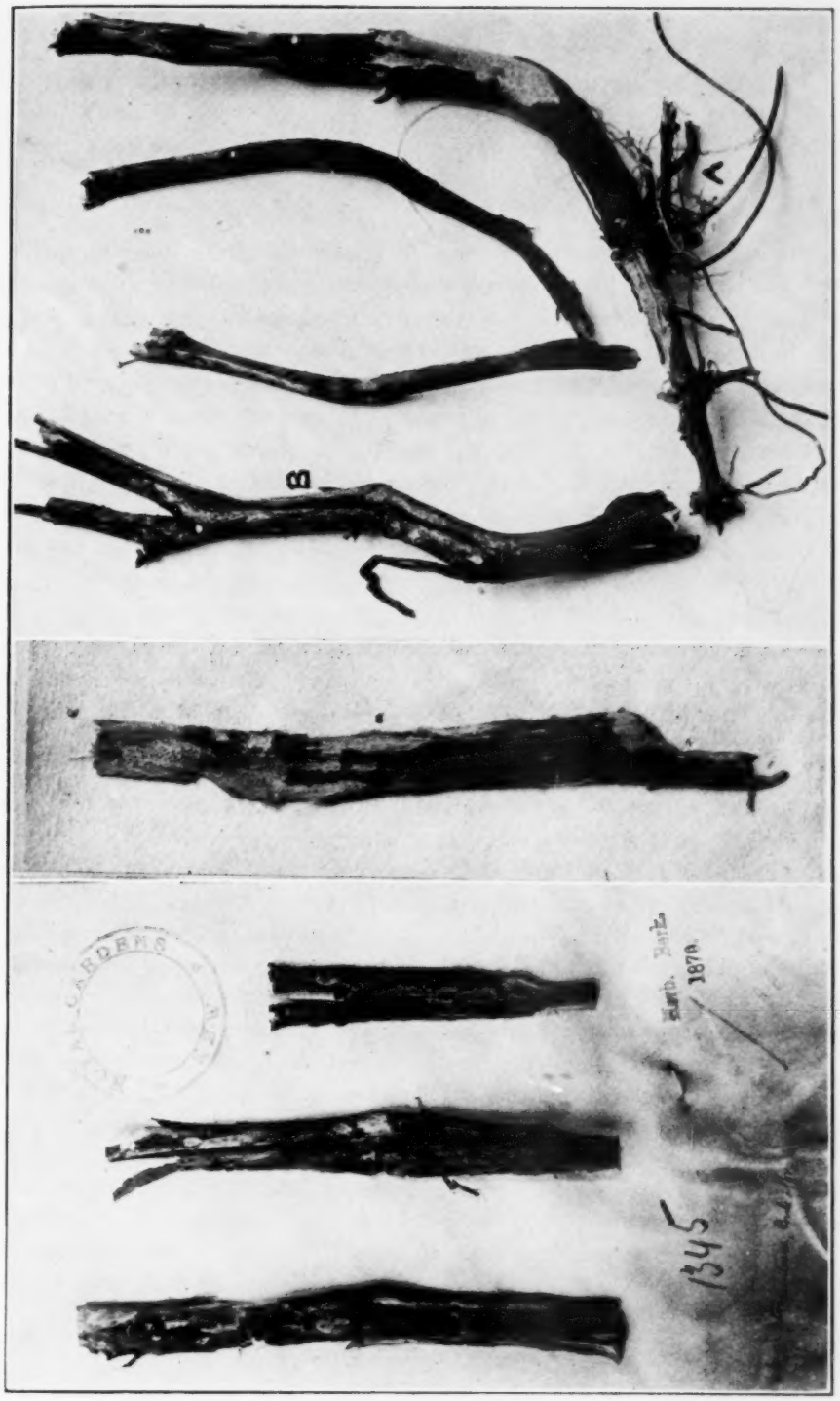
PLATE IV

1. *V. atramentaria* Berk. and Br. Note individual sclerotia in places and confluent sclerotial masses in others. Mount discolored at bottom.

2. *V. maculans* (Lk.) Desm. Closely similar to *V. atramentaria*.

3. 1923 collection of Quebec material. Decorticated tissues at A. Sclerotia confluent on stem at B.



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UNIV. OF MICH.

THE VARIABILITY IN THE BLACK ROT FUNGUS OF THE APPLE

J. S. Cooley and E. Aline Fenner

The question of the constancy of such physiological processes as the pathogenicity of a fungus is always an interesting and important one. It is particularly so with those fungi occurring in widely separated regions and having a number of unlike host plants. The black rot fungus of the apple, *Physalospora malorum* (Berk.) Shear, has a wide geographical range, for it occurs about as far north in Canada as the apple is grown and as far south in the United States as southern Georgia. It attacks the fruit and leaves of the apple and other pomaceous plants and lives as a saprophyte on the dead twigs of a great many other plants (4, 5). Since this fungus lives and thrives in unlike environments and on many different hosts, it is especially fitted for a study of variation in pathogenicity. This paper reports data on the behavior, when inoculated into the apple, of different cultures of the black rot fungus from the same locality and also from different and widely separated regions. It also reports data on the ability of the black rot fungus to produce rot after having been grown in culture for one year.

While engaged in the work reported by Brooks and Cooley in an earlier paper (1), there was some evidence that *Physalospora malorum* from different sources exhibited variability in its capacity to rot apples. It was not possible to get any further evidence on this point at that time. In the summer of 1923 experiments were begun to determine: (a) whether different cultures of *Physalospora* from the same locality differ from one another, and (b) whether *Physalospora* from one locality differs from that from another. In this way, fungi that may have been living for a number of generations in a certain environment may be compared with those from a distant locality where a very different environment exists.

The general impression seems to prevail among some workers with this organism that there are several strains possessing different degrees of virulence. Miss Walker (7) reports two strains of *Physalospora* that are very different pathogenically and morphologically. Brooks and DeMeritt (2) report three strains that show variability in pathogenicity and are somewhat unlike morphologically. Hesler (4) also mentions several strains that vary in their ability to produce rot.

The work of Stevens (6) shows that *Physalospora* produces a stroma on currant canes somewhat different from that produced on apple twigs. The spores, however, were remarkably constant on such unlike substrata.

In order to determine the variability of different cultures, not only from widely separated sections but also within a particular region, cultures were obtained from a number of different localities. Ten or more apples that appeared to be affected with black rot were secured from a given locality and the fungi isolated. Groups of about 10 cultures were thus obtained from Cornelia and Fort Valley, Ga.; Bentonville, Ark.; U. S. Agricultural Experiment Farm at Arlington, Va.; Middletown, Roanoke, Winchester and Covesville, Va.; Rockville and Snow Hill, Md.; Salem, Ind.; Mont Alto, Pa.; Durham, N. H.; and Guelph, Ontario. In some cases we were unable to obtain as many as 10 cultures from a locality. Of the 155 cultures that were used in the inoculations, 97 were isolated in 1923 and 58 in 1924. All the cultures were kept under the same environment after isolation. Isolations were made by slicing off a portion of the apple through the sound and diseased tissue and removing a bit of the decayed pulp from the margin of the rot.

The fruit for inoculation was washed thoroughly, disinfected in a bath of 50 per cent ethyl alcohol, and then rinsed in sterile water. Uniformity in the fruit of the different lots was secured by selecting as many apples of a certain size and degree of ripeness as there were cultures, placing one in each moist chamber, and repeating this until eight to ten apples were placed in each receptacle. The apples were inoculated by inserting a bit of the mycelium in the apple by means of a spear-pointed, sterile needle. After the fruit was inoculated, a piece of wet towel paper was placed in each moist chamber to maintain a humid atmosphere. The moist chambers were all stacked on the same table in the laboratory, where the temperature ran from 20° to 26° C. Previous work (1) indicated that *Physalospora* thrives well on the apple at such temperatures. After five to seven days the apples were cut through the point of inoculation or in a plane about midway between the stem and calyx and the rot measured at the point of its greatest diameter. Inoculations and measurements were made on the same day. Each experiment was concluded when the largest rots were not over 35 to 40 mm. in diameter.

In order to compare the rate of growth when mycelium and spores were used as the inoculum, an experiment was conducted in which spores were used in one series and mycelium in the other. The results in the two series were essentially the same, although the rot started somewhat more quickly when the mycelium was used.

In 1923, Grimes apples were inoculated on October 16 and November 2, Ben Davis on December 6 and February 8, and Jonathan on December 13.

In 1924, fifty-eight additional cultures were secured and used for inoculations, making in all 155 cultures that were inoculated into Grimes apples on October 9, November 8 and November 14. The number of inoculations made in the two years was about 6,800.

The data obtained from these inoculations are summarized in the graph (Fig. 1). The length of the lines represents the relative diameter of the

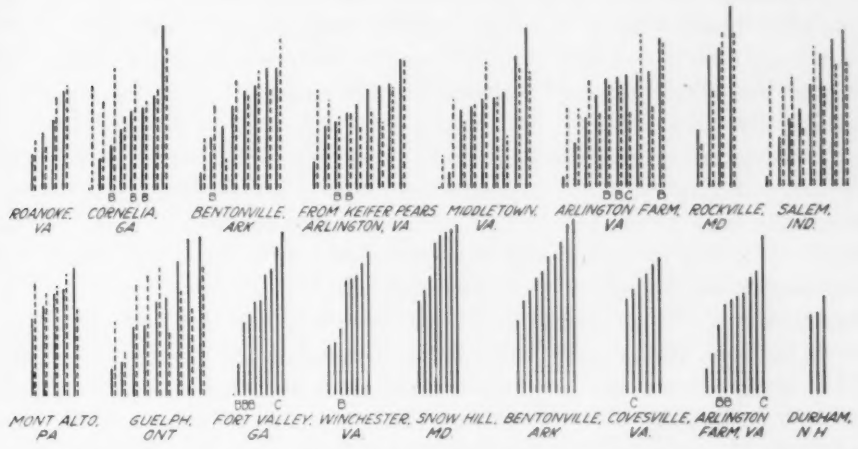

FIG. 1. The broken lines give the relative diameters of the decayed areas in the inoculation experiments conducted in 1923. Each line represents the average of three different experiments involving 8 apples each, or 24 inoculations for each fungus. The solid lines give the relative diameters of the decayed areas in the inoculation experiments conducted in 1924. Each line represents the average of 2 experiments involving 8 apples each, or 16 inoculations for each fungus. Those marked B were *Botryosphaeria ribis* and those marked C were *B. ribis chromogena*.

rot. A study of the data before the averages were made shows that there is a remarkable uniformity in the size of the rots in the apples inoculated with the same culture. It also shows that, in general, the culture that produces either a slow or a rapid decay in one experiment will behave in a similar manner in other experiments. In other words, the differences in the rate of growth of various cultures are fairly constant throughout a series of inoculations on different varieties. One might suppose, if there is a decided variation in the ability to produce rot based on environmental factors, that the cultures from Canada might represent one extreme, those from Georgia another, and that those from Maryland might lie about midway between, but a glance at the graph shows that such was not the case. The cultures from one locality were not materially unlike those from another. On the other hand, the size of the rots produced in a given time by the different cultures from the same locality showed a wide variation. In

most groups the largest rot is at least double in size, and in some cases even treble that of the smallest rot in that particular group. The cultures from one locality varied in the size of the rot produced about as much as those from another. The average diameter of the rots produced within a given time by the different cultures in many of the groups varied from about 12 to 24 mm.

The graph further shows that, with the exception of a few cultures which lost their virulence before the second year, the rot produced in 1924 was comparable in size with that produced by the same fungus when freshly isolated in 1923. The cultures were kept at about 3° C. during the summer of 1924.

The data on the graph indicate that, if a group of cultures from any one locality was large enough, it would represent a series differing from one another in only a slight degree. The range of pathogenicity in the series would be wide, the cultures at one end of the series causing a small rot with an average diameter of 5 mm., those at the other end of the series causing a rot with a diameter of 35 or more mm. A study of the graph shows that one very readily could select a culture from the middle of one of the larger groups and set it apart from those at either end of the group as being sufficiently different to be a distinct strain. This would be even more true if one took for comparison the cultures with lowest and highest rotting capacities. It is possible that, if one isolated only a limited number of cultures, he might have only those representing a low pathogenicity and those representing a high pathogenicity and thus seem to have ample ground for two or more decidedly different strains.

As will be noted, the comparison of the sizes of the rots is made on the basis of the simple diameter. This seemed to be the best method, yet it has its objections. The comparative measure of the actual rot caused by two cultures of fungi in a given time should be based on the volume of tissue decayed. If the rotted portions were exactly similar, their volumes would be to each other as the cubes of their diameters. An example of the difference in the end results between the ratio when based on the simple diameter and when based on the cube of the diameter may be cited in two cultures from Ontario, the diameters of whose rots were 12.7 and 25.3 respectively or in the ratio of about 1 to 2, while the ratio of the volumes would be 2,048 to 16,194 or 1 to 8.

In order to determine if there were any discernible morphological differences in the cultures that could be correlated with their variation in pathogenicity, the cultures were all grown on cornmeal paste and placed in the greenhouse under conditions that favored pycnidial production. By availing ourselves of the discovery of Shear, Stevens, and Wilcox (5), we

had the thrill of getting *Physalospora* to fruit at will. Both the cultures isolated in 1923 and 1924 showed the remarkable efficiency of 100 per cent fruiting. An opportunity was therefore afforded for observing the pycnidia of each culture. No sharp and definite morphological differences were detected, distinguishing the southern from the northern cultures, or the more vigorous rotters from the less vigorous.

One very interesting discovery was made in connection with the work, an account of which is given by the junior author (3). She reported that there is another organism causing a rot of the apple that is so similar to that produced by *Physalospora* that the two are indistinguishable macroscopically. Both the chromogenic and non-chromogenic forms of this species were isolated and found capable of producing a rot on apple. The cultures marked B in the graph are *Botryosphaeria ribis* G. & D., and those marked C are *Botryosphaeria ribis* f. *chromogena* G. & D. A study of the data recorded in the graph leads us to conclude that *Botryosphaeria ribis* and its related form, *Botryosphaeria ribis chromogena*, are both capable of producing a rot in the apple.

SUMMARY

1. In 1923 and again in 1924 *Physalospora malorum* was isolated from rotting apples in 14 different localities from southern Georgia to Guelph, Ontario, and from the Atlantic coastal plain to Arkansas. Groups of about 10 cultures from different fruits were obtained from each locality, making a total of 155 cultures. Inoculations were made in apple fruits in 1923 with fresh cultures, and in 1924 with both fresh and year-old cultures. The total number of inoculations was about 6,800.

2. In many cases the cultures of the black rot fungus from the same locality exhibited a wide variation in the size of the rot produced in a given time when inoculated into the apple. In some groups the average diameter of the largest rot was more than twice that of the smallest. As much variation was found within a group from a certain locality as there was between the groups from different localities.

3. *Botryosphaeria ribis* and *B. ribis chromogena* were isolated from rots resembling black rot and when inoculated into the apple produced a similar rot. Both the chromogenic and the non-chromogenic forms of *Botryosphaeria* exhibit a variation in pathogenicity that is not unlike that of *Physalospora*.

4. The rots produced by *Physalospora* and by the two forms of *Botryosphaeria* were indistinguishable.

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THE COTTONY LEAK OF EGGPLANT FRUIT CAUSED BY PYTHIUM APHANIDERMATUM

CHARLES DRECHSLER

In connection with a study of the genus *Pythium* pursued for several years, the writer has found it expedient to test out the pathogenicity of the various types encountered, by inoculating them into certain plant products obtainable on the market. An assortment including, for example, watermelons, cucumbers, squashes, potatoes, and cabbage has been found to provide a suitable variety of host materials, the reactions of which together give a general indication of the virulence of any particular organism tested. Eggplant (*Solanum melongena* L.) fruits were similarly found convenient and have been employed to some extent. They appear subject to attack in varying measure by a considerable number of strains of the type distinguished by smooth oogonia, subspherical sporangia (or "conidia"), and the production of cottony aerial mycelium, that have been assigned largely through custom to *Pythium debaryanum* Hesse. However, these forms were in all cases less energetic in their parasitism than *Pythium aphanidermatum* (Edson) Fitzpatrick. Strains of the latter, whether derived from cucumbers affected with cottony leak,[1] from watermelons affected with buff blossom-end rot, from pea roots affected with root-rot, or from dead female rootknot eelworms, *Caconema radicicola* (Greef) Cobb., uniformly proved very rapid in bringing about destruction of inoculated specimens.

That such parasitism is not confined to experimental material became evident through the receipt from C. D. Sherbakoff of a culture of a fungus isolated from diseased eggplant fruit collected by E. L. Felix in a field near Knoxville, Tennessee, September 18, 1923. According to a communication from Mr. Felix, the fungus in question had been responsible for an estimated loss of one per cent of the crop. It was readily identified as *Pythium aphanidermatum*, exhibiting the characteristic morphology of this species—numerous swollen digitate or lobulate processes that promptly develop into multiple sporangia, and sexual apparatus in considerable abundance, of which the most distinctive element is the intercalary antheridium with its dome-shaped or barrel-shaped protuberant part broadly adnate to the oogonium.

[1] Drechsler, Charles. The cottony leak of cucumbers caused by *Pythium aphanidermatum*. Jour. Agr. Res. 30: 1035–1042. 1925.

On July 20, 1925, a decay of eggplant fruits similar to that resulting from artificial inoculation with *Pythium aphanidermatum* was encountered in a number of fields in the vicinity of Bradentown, Florida. Some diseased specimens were collected and bits of affected tissue planted on cornmeal agar, with the result that 18 pure cultures of *Pythium aphanidermatum* were obtained, each from a separate, individual affected fruit. A close adherence to the morphology represented in isolations of the fungus from other sources permitted the identification of these strains with much certainty. On cucumber fruits they brought about the pronounced softening of tissue and produced the abundance of extramatrical mycelium associated with cottony leak.

The decay of eggplant fruits induced by *Pythium aphanidermatum* is an unusually rapid one, the rapidity being indicated, for example, in Plate V, A and B, representing photographs taken, respectively, 48 hours and 72 hours after inoculation by means of an incision at the blossom end. The destruction of the fruit, which measured 125 mm. in length and 110 mm. in diameter, was complete 78 hours after inoculation, at which time the extramatrical mycelium had become so profuse as to clothe the specimen almost entirely in a cottony weft. Externally the progress of the fungus becomes evident in the disappearance of the normal purple pigment, so that the affected region is distinguished by a tan coloration rather similar to that characteristic of lesions caused by *Phomopsis vexans* (Sacc. & Sydow) Harter. The zonation and surface depression distinctive of the latter parasite is absent however, the epidermis instead generally becoming minutely wrinkled, and rubbing off from the underlying tissue on very slight friction. Internally the advance of the parasite is marked by dark sepia-brown discoloration and pronounced softening in texture of invaded parts. Although, owing probably to the firmness of the outer tissues, affected eggplant fruits are at the beginning somewhat less watery than cucumbers attacked by the same parasite, in the more advanced stages a brownish liquid usually drains off in quantity. The name "cottony leak" applied to the cucumber trouble would seem to be equally appropriate here.

In the fields visited, the occurrence of the disease was confined to limited areas where poor drainage was keeping the soil in a soggy condition, especially in the furrows between the rows. All of the infected fruits were found resting with the blossom-end in contact with the moist ground, to which they were cemented, as it were, by a heavy weft of mycelium, so that, upon lifting the fruit, masses of soil were found adhering in a moldy cake. Infection in all cases appeared to have begun at the blossom end and progressed toward the stem end. No high-hanging fruits were found affected with cottony leak in any portion of any of the fields, wet or dry.

The value of adequate drainage as a method of control is clearly indicated. The season during which eggplants mature in Florida would seem generally too dry to permit the trouble to become a serious one. In the absence of pertinent information, it would seem, however, that in the more northern latitudes where rains in July, August, and September are frequently sufficient to keep the ground saturated several days at a time, losses from cottony leak might well assume proportions deserving of consideration. Owing to a certain degree of resemblance, particularly in external discoloration, of the affected fruit with fruit attacked by *Phomopsis vexans*, it is highly probable that the disease, where observed, has generally been attributed to the latter parasite. Several observers have informed the writer that instances of fruit-rot have come to their attention in which no indications of pycnidia were to be found on large well-developed lesions.

It may not be amiss to add that a species of *Pythium* of the "debaryanum" type was isolated in a single case of eggplant fruit-rot, which was found capable of reproducing the disease when inoculated into the healthy fruit. Its effect on the tissues was very similar to that of *Pythium aphanidermatum*, but the rate of destruction was approximately only one-half as rapid, and the production of aerial mycelium relatively meager. Undoubtedly, as in the similar disease of cucumbers, the capacity of the extramatrical mycelium abundantly produced by *Pythium aphanidermatum* to effect penetration through the unwounded external tissues of the host, rather than a superior degree of virulence, is responsible for the preponderance of this species in naturally infected eggplant fruit.

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EXPLANATION OF PLATE V

FIG. A. Eggplant fruit 48 hours after inoculation by incision at blossom end with pure culture of *Pythium aphanidermatum*; lower distal half of fruit tan-colored externally, and epidermis folded in minute wrinkles. × ¾.

FIG. B. Same fruit as in A, but 24 hours later; exterior entirely tan-colored, except a small proximal region at upper left-hand corner of picture; copious development of extramatrical mycelium from earliest invaded portion. × ¾.

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FOOT-ROT OF LILIUM CANDIDUM AND LILIUM PYRENAICUM CAUSED BY PHYTOPHTHORA CACTORUM

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On May 2, 1925, a diseased Madonna lily plant, *Lilium candidum* L., from an experimental plot maintained by H. E. Allanson at Tacoma Park, Md., in the immediate vicinity of the District of Columbia, was presented to the writer for examination. The specimen was submitted as illustrative of a trouble responsible for the loss of individuals of this species that had occurred from time to time during the preceding weeks. Absence of any pronounced premonitory symptoms in the foliage or in the aerial portion of the stem was reported to be a characteristic feature, the disease generally becoming apparent rather suddenly in a falling over of the entire structure above the ground. Once felled in this way, the affected individuals soon withered and died.

On examination of the specimen, the seat of the malady was readily located in the subterranean part of the stem, which, beginning somewhat abruptly at a level approximately with the ground line, was shrunken to a part of its normal diameter. The parenchyma external to the strands of conducting elements was completely collapsed. Both in this tissue and in the central pith, the microscope revealed an abundance of non-septate intercellular mycelium provided with diverticulate protuberances. Pieces of affected tissue from the shrunken region as well as from the marginal zone bordering on the healthy aerial part of the stem were placed on corn-meal agar plates. From each of the pieces the same fungus was thus isolated in pure culture—a species of *Phytophthora* producing little aerial mycelium, but an abundance of sporangia and oospores.

On May 28, 1925, a specimen of *Lilium pyrenaicum* Gouan was submitted, which had been obtained from the same plot as the Madonna lily, and which had succumbed evidently to the same disease. Except that the healthy portion of the stem extended somewhat below the ground line, the macroscopic as well as the microscopic findings were entirely similar. And from all platings of diseased tissue the same species of *Phytophthora* was again isolated.

The parasite from the two species of lily was grown in Petri dishes on various culture media, parallel with species of *Phytophthora* with which its morphology suggested a possible relationship. A high degree of similarity

with respect to rate of growth and general appearance of thallus to the species most commonly known under the binomial *Phytophthora cactorum* (Lebert & Cohn) Schroeter at once became obvious. This similarity was equally pronounced with reference to microscopical features. Measurements of the diameters of 25 oospores gave an average of 26.4 μ for the fungus isolated from *Lilium candidum*, and 27.1 μ for the form from *Lilium pyrenaicum*; while an equal number of measurements of length and breadth of conidia gave as averages for the two strains 35.7 x 27.3 μ and 34.9 x 27.9 μ respectively. These figures show a satisfactory agreement with the averages obtained from comparable measurements of the several strains of *P. cactorum*, including one obtained from the Centralbureau voor Schimmelculturen at Baarn, Holland, another isolated from rhubarb affected with foot-rot by W. S. Beach, and four strains isolated from diseased apples originating in different localities in the United States. The lily fungus is therefore referred to *Phytophthora cactorum*, thus adding two additional hosts to the list of plants attacked by this parasite.

In this connection, mention may be made of a serious disease of the Easter lily, *Lilium longiflorum* Thunb., reported by H. H. Whetzel[1] as prevalent in Bermuda in 1921, which was designated as "stump rot" and attributed to an unnamed species of *Phytophthora*. According to the account of the Bermuda disease:

> "The pathogene is harbored in the soil. Spores produced at the surface of the soil are splashed by falling rain into the crowns of the plants. Here a rot is rapidly set up which may destroy the entire stalk down through the bulb. Or only the growing tip may be killed leaving a stump with a rosette of leaves. . . . Attacks shortly before flowering are common. In these cases the tip of the stalk alone is usually killed."

The symptoms described evidently differ widely from those distinctive of the trouble that prevailed at Tacoma Park in the spring of 1925, the latter being perhaps most similar to those associated with foot-rot of tomatoes, attributable to *P. cryptogea* Peth. & Laff.[2] Future observations may determine the extent to which the relatively dry season during which the American disease developed may have affected its severity or modified its character. It is scarcely likely that the difference in specific identity of the congeneric hosts could have provided a factor of much importance, since the relationship between the Easter lily and the Madonna lily is usually

[1] Whetzel, H. H. Report of the pathologist for the period June 10 to December 31, 1921. Bermuda Repts. Bd. & Dept. Agric. 1921: 30–64. 1922.

[2] Pethybridge, G. H. and H. A. Lafferty. A disease of tomato and other plants caused by a new species of *Phytophthora*. Sci. Proc. Royal Dublin Soc. **15**: 487–505. 1919.

regarded as a close one—considerably closer than that between the Madonna lily and *Lilium pyrenaicum*. It is not impossible that lilies, like certain other hosts, may ultimately be found subject to attack by more than one species of *Phytophthora*, each responsible for symptoms revealing a measure of correspondence to, or of distinctiveness from, those induced by the others.

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OVERWINTERING OF BACTERIUM PRUNI

H. W. Anderson

During the past five years the writer has frequently attempted to isolate *Bacterium pruni* E. F. S. from cankers on peach twigs and from the buds during the dormant period, since he felt that Rolfs[1] had presented adequate proof that the bacteria wintered in these situations. Repeated failures in these attempts, together with the observations on the scarcity of cankers in many badly infected peach orchards, led to the conclusion that possibly other methods of overwintering might prevail under Illinois conditions. During the past winter, therefore, many isolations were attempted from all sources which reasonably could be expected to harbor the bacteria.

As a result of these isolations it has been established that the bacteria are able to overwinter in the dead leaves of the peach. No bacteria resembling *B. pruni* could be isolated from buds, even when these buds were obtained from twigs which had borne heavily infected leaves the previous summer. Cankered twigs were obtained throughout the dormant period from many localities in Illinois and other states, and several hundred attempts were made to isolate *B. pruni* from them, with uniformly negative results. No attempt will be made in the present article to present the negative results, although they are considered of value, and, if confirmed by another season's work, will be published in detail.

Rolfs[2] states that "cankers on the twigs and limbs are the principal sources of infection in the spring" and that "the tissues of the bud may likewise become invaded and are often responsible for the early spring infection." In regard to overwintering of the bacteria in leaves, he states: "Old leaves on the ground, if well protected from sun and air, may possibly harbor the organism for some time, but it is doubtful whether they constitute an important source of infection." He was able to isolate the organism in the spring from inoculated twigs which had died during the winter. He does not state that he ever isolated the bacteria from naturally-formed cankers after the first year. He isolated bacteria from the buds in August but gives no proof that they live there over winter. He attempted to make isolations from dead leaves but was unsuccessful except when they were left between blotters in the laboratory from October to June of the following year.

[1] Rolfs, F. M., A bacterial disease of stone fruits. N. Y. (Cornell) Agr. Exp. Sta. Mem. 8: 381–436. 1915.

[2] *Ibid.*, p. 413–415.

Roberts[3] states: "The twig lesions are of importance, however, in that in these the causal organism passes the winter."

While Lewis[4] states that the cankers on plums remain in evidence for several years, he made no isolations except from fresh cankers.

No other definite statements as to the method of overwintering of the bacterium on the peach are found in the various publications on this disease. However, it seems to be generally accepted by pathologists and horticulturists that initial infection results from the overwintering cankers.

OVERWINTERING IN LEAVES

In 1922, isolations were made early in the winter from leaves from beneath peach trees. It was extremely difficult, however, to find entire leaves in the spring, and, due to lack of experience, no spots could be identified with certainty on the small fragments which were found. Leaves placed in wire screens were completely disintegrated before spring.

During the winter of 1924–25, further attempts were made to secure cultures from leaves. Work was started as soon as the leaves began to fall, and isolations were made at least once a week until December. From the latter part of December until the opening of the buds in the spring, isolations were attempted only at intervals of two or three weeks.

Weather conditions in 1924–25 were very unfavorable for the overwintering of the bacteria. October was extremely dry, allowing the foliage to become thoroughly dried. In November several rains soaked the foliage, and continued mild weather favored decay. In December wet weather prevailed in the early part of the month, with temperatures as high as 60° F. A very heavy sleet storm on December 17 and 18 covered all exposed objects with ice. On the 20th the temperature fell to below zero F., and zero temperatures prevailed until the 30th. On the 28th, the temperature went to 20° below zero. The ice glaze continued throughout January, but warm weather prevailed in February and March. One would expect that only a few of the bacteria could survive these extreme conditions.

Successful isolations were made on January 16, February 25, March 5, and April 2, from leaves from the orchard at Urbana, and on March 10 from leaves from an orchard in Johnson County in southern Illinois. In contrast to the abundance of bacteria in platings from the leaves, platings from twigs and buds showed only a few yeasts or other fungi.

INOCULATION EXPERIMENTS

On June 5, 1925, four cultures were used to inoculate leaves of Elberta peach in the University orchard, Urbana. The first two were isolated from

[3] Roberts, J. W., Control of peach bacterial spot in southern orchards. U. S. Dept. Agr. Bul. 543. 1917.

[4] Lewis, I. M., A bacterial canker of plum twigs. Amer. Micros. Soc., Trans. 31: 145–149. 1912.

green leaves from Tunnel Hill, Illinois, on June 1, 1925, while the other two were isolated on March 5 from dead leaves collected at Urbana. The first cultures, therefore, represent recent isolations from living leaves, while the second series represents bacteria overwintering on old leaves and kept on artificial media for three months. Weather conditions during April, May, and the first week in June had been extremely dry, with practically no rain. Consequently no natural infection had taken place in the orchard.

On June 19 there was no trace of natural infection of other trees in the orchard, but abundant infection was observed on the leaves of two twigs inoculated with the recently isolated cultures, while lighter infection was noted on the leaves of two twigs inoculated with one of the cultures from dead leaves. On June 21, infection was observed on the leaves of all the inoculated twigs. The position of the spots and the general nature of the infection indicated strongly that the infection resulted from the artificial inoculation. No spots were found on the checks nor any other part of the tree, and none had appeared by July 15.

On June 25, a few cases of natural infection were found on other trees in this orchard. These were extremely scattered and only one or two infected leaves could be found on each tree. Only five trees in more than a hundred examined showed these scattered infections.

Isolations were made on June 19 and June 20 from several of the leaves from both series, and in every case typical pure cultures of *B. pruni* were obtained. This completed the proof of the identity of the organisms isolated from the dead leaves. Other inoculations both on leaves and young shoots were subsequently made with the same results.

SUMMARY

The methods of overwintering of *B. pruni* have been studied over a period of five years.

While it seems to have been established by others that the bacteria are capable of overwintering in cankers, the evidence from a study of the commercial peach orchards of Illinois would indicate that this is not the common source of initial infection.

No great difficulty has been experienced in isolating the bacteria from dead leaves in the spring, and consequently it would seem that these bacteria might be responsible in some cases for the initial infection.

Just how inoculation is brought about is not known, but, since the bacteria occur in rather large, gelatinous masses in which they are protected from desiccation, it may be assumed that these are blown as dust particles or in fragments of leaves to the new foliage and, when moistened, readily cause infection.

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BOOK REVIEWS

Éléments de Pathologie Végétale, Appliquée a l'Agronomie et a la Sylviculture. By E. Marchal. 312 pages, 148 figures. Jules Duculot, Editeur. Gembloux. 1925.

The science of plant pathology is rapidly growing through the activity of research centers in Europe, and, as the author properly remarks, also through the marvelous organization of phytopathology in the United States.

On account of the rapid accumulation of new literature and because of the scarcity in the French language of books covering the field, the author has compiled a volume embracing the diseases of farm crops, fruits, and forest trees in Belgium. A career of thirty years in that country as plant pathologist to the State has given Professor Marchal full opportunity to gather material for the book. The author does not neglect mentioning the important farm and forest crop diseases in other countries; he pays some attention to the more outstanding work done within recent years in the United States, and likewise writes on the diseases of cultivated plants in the tropics of the Belgian Congo.

It is always with interest that a new book on plant diseases is examined. A number of questions arise in regard to the organization, method of approach, point of view, emphasis, and disposition of controversial topics; consequently one is led promptly to see what disposition has been made of these matters.

The author recognizes that phytopathology, in the larger sense, deals with all phases of diseases of plants. These diseases, he states, are on the one hand due to parasites (parasitic diseases), and on the other are produced through the actior of unfavorable factors of environment (non-parasitic diseases). Unfortunately, the author has failed to take advantage of a more accurate usage, as have a number of American pathologists, in that he uses "physiologic diseases" synonymously with non-parasitic diseases. This type of oversight, however, is not the rule in this book.

Agents of parasitic diseases belong, he says, to the plant or to the animal kingdom. In many countries plant pathology is restricted to plant diseases of vegetable and non-parasitic origin. Thus, feeling that the science is a phase of botany, he leaves the parasitic diseases of animal origin to the entomologists. It is in this more restricted sense that he uses the term plant pathology.

This book is another example of a work in which the subject is approached through the field of mycology and bacteriology. The table of contents is a clear index to his manner of approach to the field of pathology. The point of view is obviously one of economic mycology. It is true that in the field of plant pathology he recognizes such phases as symptomatology, etiology, hygiene, prophylaxis and therapeutics. No apparent effort is made to explain symptomatic features through a study of infection; no attempt is made to link etiology with control; in other words, the book deals only to a minimum degree with analytical plant pathology. The author seems rather more interested in descriptive pathology, explaining for each disease discussed something of its appearance (diagnosis), brief life history points where a parasite is the cause, economic aspects, striking environmental factors, and control. And further, it seems probable that Professor Marchal attempts through this book to stimulate particular interest in plant diseases. He mildly laments that hitherto phytopathologic questions have not aroused the interest in Belgium which they merit. He finds consolation in part, however, through the fact that there have been no outbreaks of plant diseases in Belgium sufficiently serious to

arouse public opinion. Attention is called to the utilitarian character of plant pathology, public and professional recognition of which is emphasized through phytopathological inspection service, extension service, conferences, congresses, the press and scientific periodicals.

The author devotes a chapter to parasitism and parasites. He approaches the concept of parasitism through a brief excursion into the field of plant physiology. He defines virulence as that property which distinguishes parasites from saprophytes in their ability to attack living tissues. This characteristic lies, he holds, in the ability which certain organisms have of secreting substances capable of breaking down the means of defense of the host. These substances he classifies as enzymes and substances comparable to the toxins of pathogenic bacteria. Virulence may be fixed (constant) or variable; in obligate (absolute) parasites this property is constant, while in faculative forms it is variable. Through nutritional means, organisms may be induced to exalt their virulence or they may suffer loss of virulence. Parasites, depending on their restrictive host-habits, are termed polyphagus and isophagus. Immunity results, the author explains, when resistance (host defense) is victorious over virulence to the extent that the host is refractory to the infection. It is pointed out that in plants the subject of immunity has been largely neglected. Mechanisms comparable to phagocytes do not seem to exist, and while hyphae are observed to be englobed and digested, yet nothing similar to antitoxins is implied. Host-defense is classified as mechanical (anatomical) and chemical (physiological).

The specific plan of the book includes Part I, dealing with parasitic diseases of vegetable origin, and Part II, "Les Maladies physiologiques."

The general introductory chapter deals with (a) parasitism and parasites, and (b) means of control, including brief, general discussion of legislative measures, cultural measures, prophylactic and therapeutic measures. Discussion of specific diseases begins with chapter two, in which about eight pages are devoted to bacterial diseases such as blackleg and scab of potato, fire blight, olive knot, and bacterioses of beet, cabbage and tomato. In the section on myxomycetous diseases, less than two pages are devoted to club root, with probably one hundred and fifty words on powdery scab of potatoes. Fungous diseases occupy more than half the book. Two paragraphs are given over to a general statement on Phycomycetes, followed by one-half page on Peronosporales. Less than one-half page is devoted to *Pythium de Baryanum*, while *Pythophthora infestans* is treated less sparingly (six pages, more or less). Due proportion is given over to ascomycetous diseases.

Apple scab is described in the following order: symptoms, etiology (largely morphology of the fungus), nature of losses, environmental influences, and means of control. Destruction of old leaves, fruits and twigs is advised. It is also recommended that affected twigs be cut off when pruning. It is advised that the trees be sprayed with strong Bordeaux mixture before buds open, again at the petal fall, and once again fifteen to twenty days later. In cases where one wishes to give the minimum treatment, the dormant spray may be omitted, and only the application after pollination should be made. The quantities of spray mixture for various sizes of trees are specifically given (*e.g.*, 25–30 liters for a tree 8 meters crown diameter; for a hectare, 1,500–3,000 liters). Smuts, rusts, polypores and Fungi Imperfecti are more or less proportionately included. Just why the author skips over the *Fusarium* problem is not clear. Loose smut of wheat is evidently not serious in Belgium, since the author passes over the seed treatment by mere reference; the method to be used is not described. He advises, for Belgian conditions, the selection of healthy plants for seed, and, where safer methods against infection are desired, it is recommended that the heads be protected by enclosing them in gauze.

In a section dealing with black stem rust of cereals, the work of Stakman and his colleagues on physiologic forms is not wholly overlooked, yet full discussion is lacking. Practically nothing is said regarding large scale eradication of barberries; he merely advises the destruction of aecial hosts when their immediate presence manifestly influences the abundance of rust.

Filterable virus diseases are treated in a chapter set as an appendix to the study of parasitic diseases. The seven or eight pages devoted to these diseases are interestingly written, although somewhat lacking in completeness. The characteristic feature of filterable virus diseases is stated: a group of diseases presenting the remarkable peculiarity of being transmissible from a diseased to a healthy plant by inoculation or graft, without one's being able to detect in the infected tissues the presence of a causal organism. He hardly accepts the ultramicroscopic-bacteria theory, although he does not fully reject the living organism hypothesis. Mosaic and other degeneration diseases of potato, tomato, and sugar-cane are specifically discussed, while mere mention is made of these diseases affecting other crops.

The second part, which treats of non-parasitic diseases, considers the soil, its physical properties, chemical composition, effect of lack of water, excess of water, and nutritive features. Light, cold, rain, snow, hail, wind, lightning—all are given some attention in so far as they are concerned with the subject at hand. Wounds and regeneration of organs are briefly touched.

A key of some twenty pages for the determination, by external characters, of the more common diseases is probably commendable as an incentive for someone else to do better. One is only reminded of the necessity of much further study on symptomatology as such, before valuable analytical keys can be constructed.

The book is of value as an index to crop diseases in Belgium. It seems doubtful that it can be useful as a text: it is too superficial for class use, and principles are largely obscured or not mentioned. For the average student, references to literature are most valuable; yet the author has cited no references whatever.

L. R. HESLER.

Winter Botany. A companion volume to the author's *Plant Materials of Decorative Gardening.* By Wm. Trelease. Second Edition, Revised. XLII + 396 pages, 328 figures. Published by the Author, Urbana, Ill. 1925.

The second edition of this handy little volume bears the same title as the first, published in 1918, and remains, as stated in the introduction, essentially unchanged except for a few corrections and the addition of two genera (*Casuarina*, p. 9; and *Pyrularia*, p. 45).

The text deals with a total of about 1,100 species and varieties belonging to 328 genera in 94 families. The arrangement of families is according to the system of Engler. This is a desirable feature, since it avoids the confusion of being at variance with the standard floras and manuals of the country. From the standpoints of taxonomy and nomenclature, the work is conservative, and the user does not need to grope around among unfamiliar generic names nor puzzle over hair-split species.

The volume is profusely illustrated with marginal figures showing the essential details of leaf scar, buds, pith, and other important characters. Since descriptions are wanting, except for genera, these illustrations are indispensable for the correct identification of species, and because of their uniform excellence they constitute an outstanding feature of the book. References to literature on winter characters of individual species are cited after most of the generic descriptions, and in many cases valuable

observations and comments are added. A brief bibliography and a comprehensive glossary are supplied at the end of the text.

Bracket keys are used throughout, and it is hoped that their employment commended itself to the author rather from the necessity of the small pages than from choice. The key to genera runs through 30 closely printed pages with over 468 contrasting dichotomies. A bracket key of such length, compelled as it is, because of the nature of the material, to use over and over again identically worded contrasts such as "leaf scars raised—leaf scars low; twigs slender—twigs stout; buds superposed—buds not superposed," is inherently confusing and is difficult even for the experienced to manipulate.

The reviewer cannot help but feel that the value of the book, especially for the use of the non-professional botanist, would have been greatly enhanced if the monotonously long bracket key to genera could by some device have been broken up into a number of distant major divisions, or, better still, if an indented key had been constructed instead. If mechanical difficulties alone necessitated the use of a distinctly inferior type of key, it seems that a better plan would have been to reduce the scope of the work and deal with the material covered in separate parts or volumes, one for each of the two or three major floristic regions into which the 1,100 species fall. The attempt to treat such a floristically heterogenous region as the United States and adjoining Canada in a single handbook must inevitably result either in serious abridgement of the means and methods of classification, or, on the other hand, in a cumbersome product.

If it is maintained that the work deals primarily with decorative species, removed from their respective floristic regions, the fact nevertheless remains that numerous forms are included which thrive out of doors only in the extreme South, Southwest, or in California, and, if cultivated at all in the colder parts of the country, they are grown under glass, in which case flowers and fruits are frequently available in winter.

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ABSTRACTS OF PAPERS PRESENTED AT THE SEVENTEENTH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, KANSAS CITY, MO., DECEMBER 29 TO 31, 1925

The field survey as a basis for the phenological interpretation of the plant disease epidemic. L. R. TEHON.

From quantitative records accumulated by the State Natural History Survey in its plant disease survey of Illinois, those of three selected diseases—potato late-blight, wheat leaf-rust, and apple bitter-rot, are briefly tabulated and the prevalence and intensity of each correlated with temperature and rainfall by means of climographs.

From Martin's data on late-blight we may construct climographs showing July temperature and rainfall combinations productive of "severe blight," "light blight," and "no blight." Normal July temperature-rainfall combinations for certain localities in the northern and southwestern Illinois potato districts occur outside of the limits for blight. The interpretation that both of the Illinois potato regions have temperature-rainfall combinations not favoring late-blight development is substantiated by field records, blight never having been found in the southwest and only rarely in the north.

Similar climographs, based upon field data from Illinois, indicate correlations between the intensity of leaf-rust and bitter-rot infection and temperature-rainfall combinations. With adequate field data at hand, this method will measure phenological influences with an accuracy permitting considerable practical use.

The bioluminescence of Omphalia flavida, a leaf-spot fungus. A. H. R. BULLER and T. C. VANTERPOOL.

Maublanc and Rangel at Rio de Janeiro found the fruit-bodies of *Stilbum flavidum* Cke. and an *Omphalia* on spotted leaves of *Eriobotrya japonica*, etc., kept very moist; and they concluded that the *Omphalia* was the perfect stage of the fungus. S. F. Ashby, in a recent number of the Kew Bulletin, has proved this connection by the pure culture method, his isolations having been made from *Stilbum* heads produced on spotted coffee leaves. The senior author, using sub-cultures of Ashby's material, discovered that the mycelium is luminous in the dark. In further work the authors have obtained typical leaf-spots on wounded and unwounded *Bryophyllum* leaves and on wounded *Oleander* and *Ficus* leaves. The leaf spots were faintly luminous in the dark. About ten days after inoculation typical saffron-yellow so-called *Stilbum* fruit-bodies began to develop at the surface of the infected leaf-areas; and, after another ten days, at the edges of the same areas, a few typical *Omphalia flavida* fruit-bodies made their appearance. The mycelium is luminous on bread, oat-meal agar, and malt agar. The fruiting structures of both kinds give out no light whatever. No other parasitic fungus is known to produce luminous leaf-spots. Luminous leaves have been produced artificially for the first time.

Organic mercury compounds for the control of loose smuts of wheat and barley and barley stripe. I. L. CONNERS.

One-hour soak in Uspulun (0.25 per cent solution), Germisan (0.25 per cent solution) or Semesan (0.3 per cent solution) at room temperature, gave practical control of stripe (*Helminthosporium gramineum*) in Minsturdi barley. Germisan seemed the most effective. Eight per cent of stripe developed in the check plot. The modified hot-water treatment failed to control (3.5 per cent).

Loose smut in Kota wheat and Junior (hulless) barley was not controlled by the above solutions of organic mercury compounds at room temperature. Partial control, especially of loose smut of barley, was obtained with the solutions at 45° C. A presoak in water for one hour increased the effectiveness. Germisan was most effective, but it injured the germination of the barley seed. The modified hot-water treatment eliminated the smuts. The check plots showed over 20 per cent in the wheat and nearly 10 per cent in the barley.

Preliminary experiments on the control of leaf and stem rusts of wheat by sulphur dust. D. L. BAILEY and F. J. GREANEY.

Two series of fortieth-acre plots of Marquis wheat at Winnipeg were dusted fortnightly, weekly, semi-weekly, and tri-weekly with sulphur dust. The rate of application of sulphur was 15 lbs. per acre in one series and in the second series 30 lbs. per acre. The plots dusted once every two weeks were rusted as heavily as the checks. One application per week had some influence, and two applications per week had a very marked influence in controlling rust and increasing the yield. Three applications per week at 15 lbs. per acre practically controlled both leaf and stem rust, and the increase in yield was 34 bu. per acre as compared with the average of the check plots. One plot was dusted at the rate of 15 lbs. per acre when rain seemed imminent (7 applications). The percentage infection and yield in this plot were about the same as in the plots receiving two applications per week or a total of 17 applications.

Second report on dusting for cereal rusts. C. V. KIGHTLINGER and H. H. WHETZEL.

The second season's results in dusting with sulfur for the control of rusts on wheat and oats confirms the indications of the preliminary experiments of 1924. They show that proper applications of dusting sulfur will control these diseases to a significant degree.

The total increase on twenty rod-square plots of winter wheat affected with leaf rust only, was 18.5 per cent or 6.6 bushels per acre. The maximum increase on any individual plot was 47.2 per cent or 17.1 bushels per acre, while the minimum increase was 6.8 per cent or 2.7 bushels. The dusted plots were arranged checkerboard fashion, with 20 checks of the same size. Infection was reduced on the average, 48.2 per cent.

The total yield on twenty plots of 2 square rods each of oats, affected with both leaf and stem rust, was increased 19.6 per cent or 8.6 bushels per acre. The maximum yield on any individual plot was increased 35.1 per cent or 19.6 bushels per acre, while the minimum increase was 6.6 per cent or 2.6 bushels. The dusted plots alternated in checkerboard fashion with 20 equal sized checks. Infection was reduced, on the average, 37.9 per cent.

There is some indication that excessive applications of sulfur dust may cause decreased yields in wheat.

Effect of sulfur dust on the development of black stem rust of wheat in a natural epidemic. E. B. LAMBERT and E. C. STAKMAN.

Wheat was dusted in several localities in Minnesota in 1925 with precipitated sulfur at the rate of about thirty pounds per acre, to determine the effect on stem rust. A heavy natural epidemic obtained in most instances. The results support Kightlinger's statements that sulfur dust controls rust under certain conditions. Time of application is a most critical factor. In some experiments, one application virtually controlled rust in spite of a heavy natural epidemic. In others, five applications were practically ineffective, although the first application was made in advance of appreciable rust in the field. Apparently the sulfur is not effective very long after its application. Evi-

dently it must be applied just before a spore shower. It does not seem to inhibit development of uredinia after plants are once infected. In the hard red spring-wheat region, in years when there is a heavy epidemic, it would be necessary to begin dusting when the grain is in flower, or earlier, and continue until the hard dough stage, thus necessitating five or six applications. No two seasons are alike, and it is doubtful whether the proper time could be predicted with even approximate accuracy in any given season. (Cooperative investigations by the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, and the Minnesota Agricultural Experiment Stations.)

Sweet corn seed treatment in 1925. C. S. REDDY, J. R. HOLBERT, and A. T. ERWIN.

All treatments were made at the same time at one place. Liquid treatments were for 1½ hours at 25° C. Yield data on which the following table is based were taken when the corn was in the canning stage and include only corn prime for canning.

Treatment	Location	Percentage increase or decrease in yield from seed of						
		Country Gentleman			Canadian Evergreen		Golden Delicious	All lots
		Nearly disease-free	Diplodia infected	Gibberella infected	Nearly disease-free	Diseased	Diseased	
Water-soaked	Ill.	+ 0.8	− 5.1	+ 3.8	− 1.7	−14.2	+ 1.3	− 2.7
	Iowa	− 7.1	+25.3	+ 9.2	− 4.2	− 6.7	−15.3	− 1.9
Semesan 0.4% (soak)	Ill.	+ 3.6	+49.0[a]	+25.5[a]	+ 7.8	+ 3.9	+14.5	+15.0
	Iowa	− 1.6	+38.0	+ 1.5	− 4.2	+ 2.0	+25.8	+ 5.4
Semesan Jr. (dust)	Ill.	+ 6.3	+44.7[a]	+23.6	+ 7.8	− 8.4	+ 9.2	+11.9
	Iowa	− 1.6	+56.7[a]	+ 8.2	−15.0	+ 2.0	+21.8	+ 3.7
Uspulun 0.4% (soak)	Ill.	− 1.2	+54.1[a]	+24.8	+11.5	+ 2.0	+33.6	+17.5
	Iowa	-12.0	+30.4	+ 4.1	+ 4.2	+ 0.4	+38.7	+ 4.9
Bayer Dust	Ill.	− 4.3	+29.9	+19.1	− 4.5	− 2.5	+ 5.3	+ 5.0
	Iowa	− 7.1	+59.5[a]	+ 7.7	− 0.8	+ 2.4	+21.7	+ 7.5
Corona 620 0.25% (soak)	Ill.	− 5.5	+33.8[a]	+14.0	0.0	−13.3	+ 4.6	+ 3.5
	Iowa	− 9.3	+26.6	+ 4.6	− 6.7	−12.2	+35.5	+ 1.0
Corona 640 S (dust)	Ill.	− 3.6	+30.5	+ 3.8	− 1.7	− 1.5	+ 1.3	+ 3.4
	Iowa	− 7.1	+36.7	+12.8	−14.2	− 4.7	+12.1	+ 1.1

[a] Odds 30: 1 or greater, others less.

(Investigations by the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, with the Bloomington (Illinois) Canning Company, and the Iowa Agricultural Experiment Station, cooperating.)

The dilution method as a means for making certain quantitative studies of viruses. H. H. MCKINNEY and R. W. WEBB.

Although observations have indicated that mosaic viruses increase in plants, this assumption lacks definite proof; and, since questions have arisen concerning the supposed cultivation of a virus *in vitro*, it seemed advisable to test the first assumption experimentally.

Eight consecutive dilution experiments with tobacco mosaic from a single plant were conducted under greenhouse conditions which insured against accidental infection. Since our studies on tobacco mosaic show that infection occurs from soil, this source as well as others was guarded closely.

All dilutions of the virus were made in sterile, distilled water. By inoculating the plants of a given experiment with diluted virus from a mosaic plant resulting from inoculations with highly diluted virus in the preceding experiment, mosaic was obtained in the eighth experiment in practically undiminished amounts from virus which had passed through eight plants and which had been diluted in water equivalent to 10^{-33}. A single water dilution of this magnitude being far too great to produce mosaic, it is evident that the quantity of virus increased in the plants. The mosaic mottling was as illustrated by Iwanowski. All uninoculated controls remained healthy.

These and chemical fractionation experiments indicate that the dilution method offers an opportunity for making certain quantitative studies on the viruses.

(Cooperative investigations between the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, and the Wisconsin Agricultural Experiment Station.)

New virus disease of tobacco and related plants. JAMES JOHNSON.

Ordinarily only one virus disease of tobacco (*Nicotiana tabacum*) has been recognized in literature, namely, "tobacco mosaic." Two (or three) other viruses obtained from apparently healthy potatoes have been recently described. In addition to these, at least six others may affect tobacco and related plants. Descriptions of four of these have already been submitted to PHYTOPATHOLOGY for publication. These viruses are specific as indicated by symtomatology, differential host range, and properties. Certain combinations of these viruses may produce quite a different set of symptoms and in general appear to be more malignant than when each virus acts alone. Thus far, no evidence has been secured showing that the viruses in question can be modified fundamentally by passage through differential hosts. On account of the frequent use of "tobacco mosaic" in fundamental researches on the filterable virus problem, it seems important that the specific virus used in many of these studies should be more thoroughly described. Generally it will suffice to describe the symptoms, if any, secured on four or five well selected species together with the resistance to ageing *in vitro*. In other cases, however, additional details may need to be given in order to make a satisfactory description.

The destruction of the chloroplasts in tomato mosaic. HELEN SOROKIN.

In the green areas of living tomato leaves affected with mosaic many of the chloroplasts are still normal. As the disease progresses, the chloroplasts disintegrate. In the early stages there appear inside of the chloroplasts refractive, crystal-like bodies which may be either stationary or actively motile. Next, a blister-like swelling, which gradually increases in size, appears on one side of the chloroplast. The chloroplast gradually becomes smaller as the blister-like sphere increases in size. Finally the

chloroplast disappears entirely, and one or several of the crystal-like bodies can be seen inside of the spheres. Here they are practically always actively motile. The spheres are almost transparent, but can be seen readily after staining with very dilute gentian violet. They are especially abundant in the chlorotic areas of mosaic tissues. They are easily destroyed by certain chemicals, while the motile crystal-like bodies are extremely resistant to 80 per cent alcohol, 30 per cent acetone, and weak acids; but they are sensitive to weak alkalies. Whether these two types of bodies are causal or incidental has not been determined. Certainly, however, they are constantly associated with the destruction of the chloroplasts.

A mycetozoan found in tobacco plants with mosaic-like symptoms. PHILIP M. JONES.

An intracellular mycetozoan has been found in tobacco plants. It occurs only in plants with mosaic-like symptoms. Invaded cells become necrotic and the adjoining ones hyperplastic. Plasmodia may be found in all tissues except bast fibers and xylem.

The plasmodia have been cultured in sterilized Knop's solution from naturally and experimentally infected plants. These plasmodia give rise to amoebae or flagellates with one flagellum. Mitotic division occurs in both. Flagellates are formed also by conjugation in pairs of gametes produced from chromidia discharged by the nuclei of mature amoebae. Both amoebae and flagellates encyst. On germination, the cysts produce amoebae which fuse into plasmodia. These soon become granular and vacuolated and form free spores in a row the nuclei of which discharge chromidia preceding development of three- to sixteen-cyst spores. The cyst spores germinate and produce the amoeba limax. The organism is *Plasmodiophora tabacum* n.s. Similar organisms occur in potato plants affected with either mosaic or leaf-roll and tomato plants affected with mosaic. Work is in progress on the etiological phase of the problem.

Incubation period of aster yellows in its insect host. L. O. KUNKEL.

Transmission experiments indicate that the virus of aster yellows passes through an incubation period in its insect host, *Cicadula sexnotata* Fall. The length of the incubation period has varied somewhat in different experiments. In the case of nymphs a period of two weeks or more must usually elapse before non-viruliferous insects exposed to a diseased plant are able to transmit yellows to healthy plants. The incubation period is somewhat shorter in adult insects where it usually lasts from six to ten days. Viruliferous insects apparently never lose their ability to transmit yellows.

Inhibition of mosaic infection. O. H. ELMER.

Experiments indicate that diluting the juice from mosaic tobacco with juice from healthy plants causes an inhibitory effect on the mosaic virus. Dilutions were made by mixing one cc. filtered juice from mosaic tobacco with nine cc. filtered juice from bean, cucumber, and tobacco plants. Control inoculations were made with the virus diluted in water.

After the mixed juices had stood 7 to 14 days, inoculations of tobacco resulted as follows: Three series of inoculations with virus diluted with bean juice resulted in 6 per cent infection, compared with 100 per cent in the controls. When the virus was diluted with cucumber juice, infection was reduced 53 per cent and the average incubation period was lengthened three and one-half days. Inoculations with virus diluted with healthy tobacco juice resulted in reducing infection 60 per cent and in lengthening the incubation period six days.

When the mixed juices stood 21 days, the percentage infection and incubation period were similar to that in the controls. Such inoculations were made with mixtures of

virus in bean, cucumber, and tobacco juice that had shown marked inhibition ten days previously. It appears from these trials that healthy plant juices produce an inhibitory effect on mosaic virus, but that this effect is not permanent.

The relation of the seed-corn maggot to potato blackleg. J. G. LEACH.

The seed-corn maggot (*Phorbia fusciceps* Zett.) is an agent of dissemination, inoculation, and hibernation of bacteria capable of causing potato blackleg. Bacteria are constantly associated with the insect and are necessary for the normal development of the larvae. Bacteria do not occur inside of the eggs, but are commonly present on the surface. Virulent pathogenic bacteria are present in the puparia and emerge with the adult. Pathogenic bacteria also are commonly present in the intestinal tract and excrement of adult flies. Eggs are deposited in the soil on or near healthy seed-pieces or sprouts. On hatching, the maggots burrow into the seed-piece and inoculate it with bacteria which spread into the stem, causing blackleg. The insect has been responsible for outbreaks of blackleg in fields planted with carefully treated seed from fields known to have been free from blackleg. Control measures have not been devised.

Alternaria tuber rot of potatoes. L. O. GRATZ and REINER BONDE.

Potato tubers were inoculated by stirring them about in foliage spotted with "early blight" (*Alternaria solani*). Infection resulted when the tubers were inoculated and stored under either moist or dry conditions, the latter producing the most typical lesions. High percentages of infection were obtained under all conditions, but the best results were obtained by inoculating moist tubers and drying them before storing. Storage in paraffined cloth bags yielded less tuber infection, fewer lesions per tuber, and smaller lesions than storage in cloth bags. Tubers packed in soil and leaves had fewer spots than those stored in bags. Basement storage yielded higher percentages of infection, more lesions per tuber, and larger lesions than attic storage. Almost no infection was obtained at 25°, and the rate of development was very slow at from 5 to 7° C. The optimum temperature was approximately 15° C. Infection resulted on half grown tubers and throughout the season until after digging time, but was practically nil on tubers inoculated 20 days after digging. Bruising was an aid, but not essential, to infection. The most typical lesions developed when the tubers were not bruised. No lesions were observed on freshly dug tubers. The Spaulding Rose No. 4 was the most susceptible variety, but lesions were observed and produced on other varieties.

Some points of view on the plant virus problem. (Invitation paper) JAMES JOHNSON.

Virus diseases of potatoes. (Invitation paper) E. S. SCHULTZ.

Further studies of potato virus diseases in Idaho. (Invitation paper) C. W. HUNGERFORD and J. M. RAEDER.

Transmission of potato spindle-tuber disease by cutting-knives and seed piece contact. R. W. GOSS.

Successful inoculations for spindle-tuber transmission were made in the greenhouse in 1924 by rubbing together freshly cut surfaces of infected and healthy potatoes. In 1925, under field conditions, 40 inoculations were made by cutting healthy seed with a knife previously used to cut infected tubers, and a similar number were made by rubbing together the cut surfaces of healthy and infected seed pieces. Both the healthy checks and the inoculated seed pieces were from the same healthy tubers and were planted in adjoining rows.

The contact inoculations resulted in 47.5 per cent infection, 45 per cent questionable infection and 7.5 per cent healthy as compared with 32.5 per cent, 47.5 per cent and 20 per cent respectively for the knife inoculations. In addition to the distinct symptoms on the tops and tubers, the yield also was reduced, the average of both sets of inoculations being 153 gm. per plant for the disease checks, 602 gm. for inoculated and infected plants, 962 gm. for those considered questionable infection, and 1154 gm. for healthy checks. Cutting knives and seed piece contact can transmit spindle-tuber, and they may prove to be sources of infection in the field.

Resumé of results of recent investigations on potato diseases. (Invitation paper) FREEMAN WEISS.

Nuclear phenomena and life history of Urocystis cepulae. ALPHEUS WESLEY BLIZZARD.

The chlamydospores of *Urocystis cepulae* germinate, immediately after maturity, by the protrusion of a spherical, hyaline vesicle. This vesicle, the promycelium, buds out a varying number of hyphal branches which develop immediately into a mycelium. Conidia are not produced.

The mycelial cells (the homologue of the conidia) of the saprophytic mycelium may germinate and produce mycelia, thus functioning as oidial spores which serve to propagate the fungus in the soil. The mycelium grown *in vitro* is able to infect onion seedlings. The cells of the saprophytic mycelium are uninucleate. Conjugation of these mycelial cells does not occur.

The parasitic mycelium is intercellular. Certain of the vegetative hyphae are uninuclear, but the hyphal cells of the young sorus are all binucleate. Haustoria do not develop.

The spore-balls originate from sporogenous hyphae, each cell of which is binucleate. The definitive fertile spore takes its origin from a binucleate, centrally located cell of a sporogenous branch, and soon differentiates by a notable increase in size. The young spore dominates the surrounding cells of the sporogenous hyphae which are the primordia of the pseudo (nurse-cells) spores of the spore ball. The two nuclei of the young spore fuse. Thus the mature spore becomes uninucleate.

Infection and spore germination studies with Puccinia sorghi. M. A. SMITH.

Oxalis corniculata was found heavily infected with *Puccinia sorghi* throughout Iowa during 1925. The earliest infection observed was on April 28 and the latest on June 19. Normal aecidial infection was obtained with teleutospores of *Puccinia sorghi* on *Oxalis corniculata, O. europea* and *O. tropaeoloides*, while only pycnidial infection occurred on *O. valdiviensis*. No infection occurred on *O. cernua* or *O. rubra*.

In five trials the uredospores of *Puccinia sorghi* were placed on a dry substratum at a temperature of 25° C., and relative humidities of 90, 95, 96, 97, 97.5, 98.2, 98.7, 99.1, and 100 per cent were maintained. Of the uredospores, 54 per cent germinated at 100 per cent relative humidity; 46 per cent at 99.5; 36 per cent at 99.1; 25 per cent at 98.7; 11 per cent at 98.2, and 3 per cent at 97.5. Beginning September 15, 1924, germination tests were made of the uredospores held in the open. During September and October, 90 to 100 per cent of the spores germinated; 65 per cent in November; 12.5 per cent in December; 4.2 per cent in January; 3 to 4 per cent in February; and only a trace in March. After April 15, none of the uredospores germinated.

Changes in hydrogen-ion concentration induced by carbon dioxide in relation to the germination of spores of Ustilago levis. MARY F. HOWE.

In attempting to analyze the effect of fragments of living plant tissue on the increased germination of the spores of *Ustilago levis*, the influence of the carbon dioxide given off by the tissue on the hydrogen-ion concentration was studied.

Spore suspensions were made by placing the spores in three c.c. of tap water in Syracuse dishes. In such cultures it was ascertained that the highest percentage of germination occurred at pH 4.9. The colorimetric and electrometric methods were used in making this determination. A continuous flow of carbon dioxide liberated into the spore suspension from a Kipp generation caused the pH to vary from 6.7 to 4.9. A similar variation resulted where the source of carbon dioxide was fragments of living plant tissue.

Spore suspensions confined in an atmosphere consisting of different percentages of carbon dioxide failed to produce this optimum of pH 4.9. However, by a continuous flow of carbon dioxide into the spore suspension, this optimum (pH 4.9) was obtained as well as a high percentage of germination comparable to that noted in cultures where fragments of plant tissues were present.

Facultative parasitism of Alternaria and some other fungi. P. A. YOUNG.

Helminthosporium gramineum and many species of *Alternaria* and *Macrosporium* are facultative parasites having wide experimental host ranges. Their failure to infect many plants in nature shows that their host ranges are limited. They usually infected aseptic seedlings in tubes and leaves in the greenhouse within 2–7 days after inoculation or not at all. Mycelium generally projected from the lower sides of *Impatiens pallida* leaf spots. Red infection rings appeared below rings of *Alternaria* spores on sorghum leaves. *Diplodia zeae* caused a rot of wheat plumules. None of the dematiaceous fungi used caused rots of wounded apples. Cabbage leaves covered three days by shaded bell jars underwent great physiological changes, particularly in transpiration control. Susceptibility may be correlated with this change, for dematiaceous fungi infected many plants covered with bell jars, but few in open beds. Bell jars probably did more to augment infection than merely preventing evaporation of water placed on the leaves. *Helminthosporium gramineum* and three species of *Alternaria* acted like rust fungi in causing green spots in yellow leaves of wheat, rye, radish, and nightshade. Perhaps fungous enzymes stimulate formation or delay decomposition of chlorophyll in host cells near such leaf spots.

Infection phenomena of Alternaria, Diplodia, and some other fungi. P. A. YOUNG.

Diplodia zeae, *Cephalosporium acremonium*, *Colletotrichum nigrum*, and many species of *Alternaria*, *Macrosporium*, and *Helminthosporium* induce infected wheat coleoptiles to form spherical or cylindrical, hyaline to brown bodies on and in the cell walls. The name "callosities" has been proposed for these bodies. Their formation is a typical reaction of plumules of wheat, oats, rye, barley, pop corn, sorghum, broom corn, cabbage, radish, turnip, tomato, soybean, wax bean, pumpkin, muskmelon, and *Abutilon theophrasti* to infection by *Alternaria* and *Macrosporium*. No callosities appeared in infected cells of the membranes between onion bulb scales. Callosities partly or entirely enclose narrow penetration hyphae which often connect them to appressoria or internal hyphae. Wheat coleoptiles infected with *Alternaria* characteristically exhibit callosities, isolated or numerous brown cells, swollen cell walls, penetration hyphae, internal hyphae, and, when stained with Congo red, red discs of altered cell-wall material surrounding infection points. Such red discs were seen in wheat cells infected with *Colletotrichum* and *Cephalosporium*. *Alternaria* caused auto-stained brown discs to appear at infection points in sorghum coleoptiles. Mechanical injury alone caused brown discolorations of wheat, sorghum, and broom corn coleoptile cells. Internal hyphae of *Alternaria* occurred in swollen cell walls of wheat, cabbage, and muskmelon.

Downy mildew (Sclerospora graminicola (Sacc.) Schroet.) on the Everglade Millet (Chaetochloa magna (Griseb) Scribn.). W. H. WESTON, Jr., and G. F. WEBER.

The causal fungus of downy mildew of Everglade millet appears to be *Sclerospora graminicola* (Sacc.) Schroet. It was collected on *Chaetochloa magna* (Griseb) Scribn., growing wild in the Everglades of southeastern Florida. No other host has been found.

Its conidial stage, in size, structure and method of development of the conidiophores, in the structure, germination and quantitative measurements of the zoosporangia (conidia) and the oogonial stage in its development, structure and biometric characteristics, agree in their essential features with the representative material of the species as it occurs typically on *Chaetochloa viridis* in Minnesota and other northern states. This is the first time, however, that this species of *Sclerospora* has been found in the southern states and the first time also that it has been reported on this host in any locality.

The attack of the fungus usually results in systemic infection, but occasionally the disease has been found localized in limited spots on the leaves. During the later stages great numbers of oogonia develop in the mesophyll tissue of the mature leaves which disintegrate and become shredded out into fibrous tangles. Large numbers of oogonia also develop in the inflorescences which grow abnormally into distorted and deformed structures. (Cooperative investigations by the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, and the Florida Agricultural Experiment Station.)

Studies on Helminthosporium sacchari on sugar cane in Porto Rico. MEL. T. COOK.

This fungus was first described from Java by Van Breda de Haan in 1892 as *Cercospora sacchari*. Butler transferred it to the genus *Helminthosporium*. The species described from many of the West Indies is considered the same organism as that reported from Java, India, Hawaii, and other places. It is now considered a very destructive disease in Hawaii and Porto Rico.

The first destructive outbreak in Porto Rico was in 1923, and the second in 1925. The development of the disease is influenced by temperature and moisture. It is exceptionally destructive to young plants, killing great numbers of them in some varieties. Its sudden recognition as a serious disease probably is due to the development of new varieties, some of which are very susceptible.

Colletotrichum trifolii and Gloeosporium caulivorum on clover. JOHN MONTEITH, JR.

Investigation of the two common clover anthracnose diseases has shown their distribution largely dependent on temperature. On potato dextrose agar, the optimum temperature for growth of *Colletotrichum trifolii* is about 28° C., whereas the optimum for *Gloeosporium caulivorum* is about 20° C. The lowest favorable temperature for *C. trifolii* was 12° C., while *G. caulivorum* grew at temperatures as low as 4° C. At 32° C. the *Colletotrichum* grew rapidly but the *Gloeosporium* made almost no growth. *C. trifolii* produced spores abundantly at 20° to 32° but not at the lower temperatures, whereas *G. caulivorum* over its whole temperature range gave the typical colonies consisting almost entirely of spores produced by a process of budding, with practically no development of mycelium. Artificial inoculations under controlled conditions indicate that this temperature relationship is also similar for infection. The two fungi produce virtually the same disease symptoms on clover. *C. trifolii* causes most damage during warm weather, and is especially common in the southern states. *G. caulivorum* is usually found in northern sections or in the South during the cooler seasons. Certain imported strains of red clover are most susceptible, while certain American strains are most

resistant to both organisms. (Cooperative investigations between the Office of Cotton, Truck and Forage Crop Disease Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, and the Agricultural Experiment Station of the University of Wisconsin.)

Study of Peronospora trifoliorum DeBy. on species of Leguminosae. M. K. PATEL.

Because of increased acreage of alfalfa, *Peronospora trifoliorum* is assuming greater economic importance in the middle west. Its demonstrated host range is limited to *Medicago sativa* L. and *M. lupulina* L., although 31 species in 16 genera of legumes were exposed to infection with viable conidia of *Peronospora*. Stock cultures are maintained readily in the greenhouse on seedling alfalfa plants. In such cultures conidia and oospores are formed in abundance.

Infection in alfalfa, although usually local, may become systemic, involving parts of the shoots from a crown. *P. trifoliorum* survives unfavorable growth periods by oospores produced sparingly in the leaves of alfalfa in the late fall. Although many attempts have been made to show that the mycelium may live over in the infected plants, no positive data have been secured. Conidia placed in water and allowed to freeze were viable after 173 hours. The optimum temperature for the germination of conidia was 18° C., minimum 4° C., and maximum 29° C. The spores would not germinate on a dry slide in atmospheric humidity of 100 per cent. A film of water was essential for germination. The presence of formaldehyde, acetaldehyde, benzaldehyde, butyraldehyde, furfuraldehyde, toluol, and ethyl alcohol in the germinating chamber inhibited germination of conidia.

A yellows resistant strain of Copenhagen market cabbage, Iacope. FRANK VAN HALTERN and A. T. ERWIN.

A strain of Copenhagen market cabbage, known as Iacope, has been isolated and, in the field, has proved consistently resistant, during the past three years, to the cabbage yellows disease (*Fusarium conglutinans*). Its resistance is variable, ranging from 75 to 95 per cent, depending on the extent of soil infestation and the temperature range.

Iacope, like its parent, has a medium large, round, solid head, less fixed as to shape, color, and time of maturity. The yield of Iacope is fully equal to, if not greater than, that of Copenhagen market. In 1924, it yielded at the rate of 12 tons and in 1925, ten tons per acre. Seed of Iacope is now available through the Muscatine Cabbage Seed Association and several of the leading seed firms in the middle west. The seed-bed method of selection has permitted the building up of its resistance in successive generations. The progeny of selfed, resistant strains has proved equally or even more resistant than the parent.

A new Fusarium resistant cabbage. J. C. WALKER, JOHN MONTEITH, JR., and F. L. WELLMAN.

For some years attention has been given to the development of Fusarium-resistant strains of mid-season varieties of cabbage. The results reported herein pertain to selections made from the variety, Glory of Enkhuizen. The method heretofore practiced of making head-to-row tests from open-pollinated plants has been modified by greenhouse propagation of seed, wherein plants are selfed or close-pollinated artificially. Progress has thus been hastened. Moreover, a line has been isolated which appears to be very uniform in type and practically homozygous for resistance in the field. The evidence of stability in resistance has been shown in two successive generations on

thoroughly infested soil in southeastern Wisconsin, and has been corroborated in trials in a number of other localities. Further evidence is found in the fact that whenever individuals of this line have been crossed with susceptible individuals the F_1 hybrids show practically 100 per cent resistance in each case. Because of slight deviation in type from the standard variety, and in view of the added character of resistance, the new strain is to be given varietal distinction. Steps have been taken to increase the new variety for commercial use as soon as practicable. (Cooperative investigations between the Office of Cotton, Truck and Forage Crop Disease Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, and the Agricultural Experiment Station of the University of Wisconsin.)

Ammonia injury of fruits and vegetables in storage. G. B. RAMSEY and L. F. BUTLER.

Brownish-black discolorations of fruits and vegetables, normal in every respect except color, frequently have been observed on the Chicago market. Discolored onions have been found most often, but peaches, apples, pears, and bananas also have been noted showing a similar type of injury.

The absence of indications of a causal organism, and the consistent association with stored products, indicated that the injury had resulted from exposure to a uniformly distributed, deleterious substance while held in storage. This type of injury was duplicated, in the laboratory, with ammonia. It was demonstrated that the yellow, brown, and red pigment-bearing tissues become brownish-red to dark brown, and in some cases black, when exposed to ammonia. The extent of injury is determined by the amount of ammonia in the air and the duration of the exposure. The injury may be merely a blemish, as in the discoloration of the outer dry color-bearing scales of the onion; or it may be serious, as in the softening and browning of the peach. Tables showing the degrees of injury of the products mentioned, when exposed to known strengths of ammonia for varied time intervals, are included in the complete manuscript. (Contribution from the Research Laboratory on Market Diseases of Fruits and Vegetables; United States Department of Agriculture, Bureau of Plant Industry, and the Botany Department, University of Chicago, cooperating.)

Chemical injury to watermelons in transit. W. W. GILBERT and F. C. MEIER.

Railroad companies and shippers have frequently reported injury to watermelons caused by contact with chemicals, including fertilizer materials on the floor and walls of freight cars.

Preliminary experiments were conducted last fall to determine whether any chemicals carried as freight can damage melons and if so under what conditions and the character of the lesions. To approximate shipping conditions, both wet and dry chemicals were used. Some melons were placed directly on the chemicals, while others were separated by building-paper or a layer of straw. Pressure equivalent to that normally received in the car was applied to half the melons. After 5 days, melons under pressure showed marked injuries (discoloration, pitting, softening) when in direct contact with the following moistened chemicals: acid phosphate, potassium muriate, salt, and sodium nitrate. Some damage under the same conditions resulted from ammonium sulfate and potassium sulfate. Neither hydrated nor air-slaked lime was injurious. Slight damage occurred when ammonium sulfate and salt were used dry and no pressure was applied.

Melons under pressure, when protected from wet chemicals by layers of building paper, were not so severely affected. Where straw was used for bedding, even less injury resulted.

Further evidence of the non-transmissibility of the so-called sweet potato mosaic. JAMES L. WEIMER.

Nancy Hall sweet potatoes from vines showing symptoms of mosaic were carried through three generations under different environmental conditions, the mosaic symptoms being evident to about the same degree on each. Healthy plants, with vines growing intermingled with those of diseased plants, gave no evidence of disease even in the second generation. Unsuccessful attempts were made to transmit the disease by the usual mechanical means, as well as by grafting halves of diseased and healthy potatoes. Likewise, portions of diseased vines were grafted onto healthy roots and vice versa. The grafts, following a good growth in the greenhouse, were set in the field. The healthy vines, after growing on diseased roots for eight months, were still healthy, and the diseased vines on healthy roots remained diseased. A small crop of potatoes was produced by all of the plants. Whenever these potatoes sprouted before being dug the young plants were diseased if attached to a diseased root, and healthy if from a healthy root.

Sweet potato mosaic and its incubation period of two growing seasons. H. R. ROSEN.

In his first communication on sweet potato mosaic in 1920, the writer called attention to the non-communicability of this disease. Macerating diseased material and applying it in various ways, including rubbing of young and old leaves, hypodermic injections into stems, petioles, and midribs, heavy applications into cavities made in fleshy roots, then used for propagation, and grafting healthy shoots on diseased plants all resulted negatively. This was true irrespective of time of inoculation or age of inoculated plants. Young as well as old plants failed to show any signs of disease during the growing season. This work has now been confirmed by other investigators, and certainly sweet potato mosaic is not communicable as far as concerns the development of recognizable symptoms during one growing season.

Experiments made by the writer for several years show that the disease has a remarkably long incubation period and, even under optimum conditions, no symptoms appear in less than two successive growing seasons. Furthermore, there usually is a gradual increase in the severity of the disease in the progeny of inoculated plants, although sometimes the symptoms are pronounced when the disease first appears.

Fusaria causing bulb-rot of onions. GEO. K. K. LINK and ALICE A. BAILEY.

Several species of *Fusarium* cause bulb-rot of onions. Two hundred isolations have been made from bulbs collected in the field and market. The pathogenicity of half of these cultures has been tested, but the taxonomic position of all has not yet been determined.

The species which invariably are pathogenic belong to section Elegans. The following have been determined: *Fusarium zonatum* Sh. forma I Wr. (*Fusarium cepae* Hanz. emend. Walker and Tims); *Fusarium zonatum* Sh. forma II nova forma; *Fusarium cepae* Hanz. emend. Link and Bailey. One member of section Liseola, *Fusarium moniliforme* Sheld., isolated from decaying bulbs, is not invariably pathogenic (50% +).

Lesions vary from a mealy, vinaceous buff, to a powdery, dry (mummied) avellaneous to cinnamon decay. Not all Fusaria isolated from onions affected with bulb-rot are pathogenic. Some, when associated with bacteria, cause a decay unlike the typical bulb-rot produced by pathogenic bacteria alone, but like that produced by pathogenic Fusaria when bacteria are present. Identified species of section Elegans which behave in this way are: *F. oxysporum* Schlecht., *F. vasinfectum* Atk., and *F.*

bulbigenum Cke. and Mass. *F. martii* Appel and Wr. var. *minus* Sh. of section Martiella behave similarly.

(Cooperative investigations between the Office of Cotton, Truck and Forage Crop Disease Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, and the Botany Department, The University of Chicago.)

Pseudomonas (Phytomonans)pisi Sackett, the cause of a pod spot of garden peas. C. A. LUDWIG.

A pod spot of garden peas, first discovered in 1922, has been found due to an organism believed to be a strain of *Pseudomonas (Phytomonas) pisi* Sackett. The organism may live in culture and retain its infective capacity without being transferred for more than a year, under some circumstances at least. It can attack stems and leaves as well as pods. It seldom infects, however, except at some point of injury. It infects young, succulent tissues more readily than older, drier ones, and some varieties more freely than others. Culturally, it behaves very much like *Pseudomonas pisi*. The discovery or development of resistant varieties is suggested as the most promising approach to its control.

A wilt disease of peas in Wisconsin. MAURICE B. LINFORD.

A wilt disease of canning peas caused losses in Wisconsin during 1924 and 1925 second only to those caused by *Aphanomyces* root-rot. This newly-observed disease is characterized by early and rapid wilting of leaves with or without previous yellowing, and without decay or lesions of the cortex of roots or basal stem. It develops, characteristically, roughly circular patches throughout a given field. These appear early, and may continue to enlarge until the crop is harvested. Wilt is most frequently destructive in fields which have been heavily cropped with peas. Vascular discoloration occurs frequently but not constantly. Fungal invasion of the vessels of the taproot and lower internodes is characteristic.

Isolation of fungi from the vessels of wilted plants from 68 collections of peas from 18 counties in Wisconsin yielded cultures which fall into three groups, regarded tentatively as three undetermined species of *Fusarium*. Seventy-five cultures from one of these groups have proved pathogenic, while no pathogenicity has been demonstrated in the other two groups.

Green Admiral, a canners' variety, appears highly wilt-resistant, and certain newer varieties promise equal or greater resistance.

Aspergillus decay of cotton bolls. MICHAEL SHAPOVALOV.

A peculiar decay of cotton bolls has been prevalent during the last few years in the southwestern states. It appears at first as a soft, pinkish, circular spot and may spread over the entire boll. The latter prematurely dries up and becomes filled, and covered on the outside with black masses of spores which give it the appearance of being affected by smut. It is erroneously known as smut in some cotton-growing sections of California. The fungus causing this decay is *Aspergillus niger*. The infection is sometimes associated with insect wounds, but frequently such injuries are not apparent. Successful inoculations have been made of green-picked bolls in the laboratory, as well as of growing bolls in the field, by inserting spores of the fungus in scalpel stabs or in needle pricks. Up to 100 per cent of infection has been obtained in moist chambers and as much as 76 per cent in the field.

Cotton wilt studies. V. H. YOUNG.

Strains of *Fusarium vasinfectum* from several states showed an appreciable difference in their pathogenicity for cotton, and appeared to be distinct strains. Some proved extremely pathogenic; others much less so. Later trials showed great variations, the last ones resulting in almost no infections, apparently because soil temperatures were too low. Loss of pathogenicity in culture must also be considered as a possibility.

Inoculations with a monosporic strain of *Fusarium vasinfectum*, under ordinary greenhouse conditions during late summer, and in soil temperature tanks at 30° C. gave 50 per cent infection under the latter condition and none under the former. Experiments in the temperature tanks, using temperatures from 22½° to 35° C., gave no infection at 25°, some infections at 27½°, and the greatest number at 32½° C. There was still infection at 35° C. A second experiment gave similar results. The maximum soil temperature for cotton wilt infection is higher than 35° C. and the minimum temperature is near 25° C. Results are not definite as to the optimum point for infection.

Control of brown-patch in turf. JOHN MONTEITH, JR.

There are two types of brown-patch occurring on lawns and putting greens. Large brown-patch is generally limited to periods of hot, humid weather but the small-spot type may occur over a much wider range of climatic conditions. The effect of fungicidal treatments is different with these two diseases. Large brown-patch may be readily controlled by spraying or dusting with Bordeaux or other copper sprays. Repeated applications of copper salts, however, produce an accumulation of toxin in the soil which may seriously injure turf. Small brown-patch is checked by neither organic nor inorganic copper sprays. All the mercury compounds tested, Uspulun, Semesan, Germisan, Corona 620, Corona 640, and mercuric chloride, controlled both types of brown-patch. The period of protection given by the mercury salts varies from a few days to several weeks, depending on the amount applied, severity of disease attacks, soil, rainfall, and other environmental factors. Formaldehyde, under certain conditions, may protect grass from these diseases for a few days, but its effect is not as lasting as that of the mercury applications. Sulphur does not check either type, and is toxic to grass.

Studies on leafhopper injury to apple leaves. A. A. GRANOVSKY.

During the past three years the leafhoppers of cosmopolitan food habits such as *Empoasca mali* (Le Baron), *E. flavescens* (Fabricius) and *Cicadula sexnotata* (Fallen) were present in abundance in apple orchards of the Peninsular region of Wisconsin, the first being the most abundant. At the same time various degrees of apple leaf injury have been observed which could not be ascribed to any well known cause. It manifested itself in characteristic marginal discoloration of the leaves which gradually progressed downward along the lateral veins, often reaching the midrib. This discoloration is invariably more rapid on the basal than the apical side of the veins. In case of severe injury, the tips and the distal margins of the leaves curled upward, much resembling the "hopperburn" injury of potatoes.

Experiments with caging leafhoppers over the terminal spurs of apples indicate that this type of discoloration is due to the feeding of leafhoppers. Prolonged confinement intensifies the symptoms. Needle inoculations with the macerated leafhoppers produced similar symptoms. The sap of diseased leaves gives a relatively low percentage of infection. The sap of uninfected leaves does not produce the characteristic symptoms. It is believed that this type of apple leaf trouble is due to feeding of leafhoppers and probably to some toxic or infectious agent.

Migration and transformation of Bacillus amylovorus in apple tissue. E. L. NIXON.

The migration of *B. amylovorus* was traced from needlepoint infection to canker formation. Migration of the organism in the form of zoogloea was found in the early stages taking place through inter-cellular spaces in the cortical regions. Subsequently, the organism penetrates the walls of the phloem parenchyma and medullary-ray cells, occupying the space between the walls and the protoplast. In some cases, it further penetrates the protoplast of healthy cells and forms a cyst within the central vacuole. In this condition the organism hibernates. With renewed growth of the host in the spring, the cysts imbibe moisture and disintegrate. The bacterial masses then escape from the host cells. These masses uniting with others then appear as the familiar exudate. In tracing the migration, it was observed that in certain regions the bacterial masses appear to disintegrate. In other regions involution and spore forms occurred.

Studies of apple scab infection under controlled conditions. G. W. KEITT.

An inoculation chamber has been devised in which a saturated atmosphere can be maintained at a wide range of constant temperatures. Leaves of potted apple plants were inoculated by means of ascospores of *Venturia inaequalis* (Cke.) Wint. naturally discharged from apple leaves suspended in the upper part of this chamber. Experiments thus far performed show that the minimal temperature for infection is not more than 6° C. and the maximal not less than 26°. It is probable that further work will extend the lower temperature limit. The optimal temperature for infection was near 20° C., which agrees closely with the optimum for germination of ascospores and growth of *V. inaequalis*. When inoculated plants were held at constant temperatures, the minimal periods of incubation observed at the different temperatures varied from 17 days at 8° C. (lowest temperature tried) to 8 days at 20°.

The minimal numbers of hours of continuous wetting which have thus far yielded leaf infection by ascospores at the temperatures stated are as follows. 6° C., 18; 9°, 13; 15°, 8.5; 20°, 6; 26° 10.

Studies are in progress on the relations of temperature and moisture to the effectiveness of fungicides commonly used to combat scab.

Studies on the development of the ascigerous stage of Venturia inaequalis in nature. G. W. KEITT and E. E. WILSON.

Conditions affecting the abundance of ascospores of *Venturia inaequalis* and the time of their maturity and discharge are of much potential importance in relation to the development and prevention of epidemics of apple scab. Consequently, the development of the fungus at Madison, Wis., was studied from a time prior to leaf-fall until ascospores were matured and discharged. A marked relationship was observed between the time of leaf-fall and ascospore maturity. Under the conditions studied, ascospores matured much earlier in leaves which fell in early autumn than in those which remained on the tree until late autumn or early winter. Observations and experiments are in progress relative to the possible bearing of the time of leaf-fall upon control measures.

Temperature and moisture were shown to be factors of cardinal importance in determining the rate of development of ascocarps.

Infected leaves were sprayed with various chemicals shortly before leaf-fall in the hope of finding a treatment which would prevent or limit the development of the ascigerous stage of the scab fungus without serious injury to the host plant. The results thus far obtained offer sufficient promise to justify continuation of the experiment.

Studies of crowngall and callus overgrowths on apple grafts. A. J. RIKER and G. W. KEITT.

Isolation studies were made on 227 apple trees, representative of lots of stock submitted as crowngall rejects by 12 nurseries in six states. Less than two per cent of these trees yielded *Bacterium tumefaciens* Smith and Town. Tests of the isolation technique on known crowngall material gave positive results from 44 to 46 plants.

A study of the trees which failed to yield the crowngall organism showed most of the malformations to be associated with poorly fitted grafts. Various types of misfitted grafts were grown experimentally in comparison with well-fitted grafts. Malformations could be induced or prevented almost at will by the type of fit. An examination of samples of freshly prepared commercial grafts from eight nurseries showed misfits in sufficient numbers and types to account for all the rootknot reported by these nurseries. The following types of misfit appear to lead commonly to the more severe callus overgrowths: cion tip long; cion larger than stock; graft too loosely wrapped.

Wrapping grafts with cloth, waxed paper, or medical adhesive tape reduced the amount of callus development.

The results thus far available give promise that the major portion of the callus overgrowths at the unions of grafted apples may be prevented by modifications in grafting practice.

Aerial galls of quince. J. A. MCCLINTOCK.

In connection with crowngall studies in Tennessee, two old quince trees, showing numerous well developed aerial galls, have been under observation since the fall of 1924. Attempts to obtain the crowngall bacterium from these galls have been unsuccessful. Hard-wood cuttings made from galled branches of various ages were placed in a sand bench in the greenhouse for comparison with similar quince cuttings from younger, gall-free trees. Repeated examination of the cuttings disclosed no additional gall development, but in all cases where roots did develop on the galled cuttings they grew out from the galls. This indicates that the aerial galls on these old quinces are not crowngalls, but that they are similar to the "burr-knots" of apple and quince described by Swingle.

Hairy root of apple seedlings. J. H. MUNCIE.

Three distinct types of hairy root have been observed on French apple seedlings, namely: (1) the woolly knot form, arising from a distinct gall; (2) the simple form described by Stewart; (3) the type in which fine fibrous roots arise in clusters from the tap root.

The first type is infectious, and has been induced by artificial inoculation of healthy seedlings in steamed soil with *Bacterium tumefaciens*, and by growing healthy seedlings in soil artificially and naturally infested with that organism. The second type was the least common, occurring on seedlings grown from treated seed in steamed soil. The third form was the most common, occurring on seedlings in steamed soil as generally as in check plots receiving no treatment. The percentage of this type was not increased by infesting steamed soil with *Bacterium tumefaciens*. Repeated attempts to isolate *Bacterium tumefaciens* from type three have been unsuccessful, and histological studies have failed to show the presence of an organism within the hairy roots or near the point from which they arise. These last two types on seedlings seem to be non-pathogenic forms of hairy root.

The effect of crowngall on young apple and peach trees and longevity of Bacterium tumefaciens in the soil. J. H. MUNCIE.

Work has been continued on water conduction in galled and normal piece-root grafted two-year "cut-back" Wealthy, Jonathan, and Salome apple, and budded two-year old Carman, Salwey, J. H. Hale, and Elberta peach trees. In 206 isolations from 84 galled apple trees used in these studies, *Bacterium tumefaciens* was recovered in four cases. In 200 apple trees examined by means of longitudinal sections through the middle of the union, 81 per cent of the healthy showed a perfect union of stock and scion, while 63 per cent of the galled showed only partial continuity.

The reduction in water flow through galled Wealthy, Salome and Jonathan trees was 69.7, 21.7, and 47.2 per cent respectively. Lateral roots arising from above or opposite the gall may in some cases counteract the obstruction offered by the gall. In peach the average reduction in water flow through the galled trees is 82.4 per cent.

Young tomato plants were set in soil infested with *Bacterium tumefaciens*, and inoculated by injuring the stem at the surface of the soil and smearing the wound with the infested soil. Typical crowngall resulted up to 102 days after infesting the soil.

A wound dressing for plants. I. E. MELHUS.

A wound dressing possessing many desirable characteristics has been made from the resinous products resulting from refluxing furfural and creosol. It is made by mixing one pound each of commercial creosol and furfural (concentrated) with 0.32 ounces sodium bicarbonate in a reflux condenser and boiling until a resin forms. When it becomes slightly brittle on cooling in a thin film on a glass rod, discontinue boiling and pour the sirupy mass into a shallow pan to cool quickly. Dissolve this resin in equal parts of acetone and furfural. Dissolve four grams mercuric cyanide in a small quantity of acetone, and add it to the resin-acetone-furfural mixture.

This dilute resinous mixture, about the consistency of shellac, can be applied to a wound with an ordinary paint brush. It dries quickly, forming a smooth, dark brown, amorphous coating both on living and dead tissue. The rate of drying and penetration is determined largely by the proportion of acetone to furfural. The plastic and amorphous properties can be controlled by varying the degree of condensation of the resinous product. Dead tissues are penetrated from one to two inches by the furfural in the dressing, while living tissues are not penetrated nearly as extensively by the furfural. The resin remains largely on the surface of the wound.

Control of bacterial spot of peach with sodium silicofluoride. H. W. ANDERSON.

Spraying experiments on peach for control of bacterial spot (shot hole) during 1925 at Urbana, Illinois, demonstrated the possibility of control with a spray of sodium silicofluoride in water. Preliminary laboratory tests proved this substance effective in preventing growth in broth cultures in dilutions of 1–3,000. Seven applications were made, ten to fourteen days apart. Various concentrations were used. One containing two pounds in 50 gallons of water proved satisfactory. The checks showed from 10 to 90 per cent diseased leaves on October 1, while very few infections could be found on the sprayed trees. It is probable from the dates of infection that only three of the seven sprays would have been necessary for control. Sulfur sprays first gave increased infection over the checks, due probably to wetting the trees during spraying. Later, the checks were more heavily infected. No injury to the foliage resulted from the use of the sodium silicofluoride, neither in the orchards nor in the greenhouse where preliminary tests were made. This season's weather conditions were so abnormal, how-

ever, that no assurance can be given that injury will not result in other seasons. Copper sprays, including two brands of "colloidal" copper and Bordeaux mixture, caused serious spray injury.

Observations on masking of raspberry mosaic by high temperature. R. B. WILCOX.

Certain types of raspberry mosaic are masked during hot weather. Mottled leaves formed during cool weather retain their mottling, but new leaves developed during hot weather appear normal. Temperature-control chambers were not available to the author for a study of this masking.

Rubus innominatus, infected with one type of transmissible mosaic, was found to be not only peculiarly sensitive to temperature changes, but recorded these changes by very conspicuous leaf markings. Such infected canes, accompanied by a record of their growth during the summer, showed correlation between the appearance of seemingly healthy leaves and the occurrence of periods of high maximum temperatures. Study of this relationship indicates that for this mosaic of *Rubus innominatus* the critical temperature is approximately 75° F. (24° C.).

A citrus bacteriosis occurring in South Australia. H. K. LEWCOCK.

Intermittently during the last decade, particularly in wet seasons, a bacterial disease of citrus fruits has manifested itself in the fruit-growing districts of South Australia and Victoria. In some instances, losses amounting to 30 per cent to 40 per cent of the crop have been recorded. Such heavy infection, however, has been restricted to districts with moderately heavy winter rainfall, *viz.*, 20 to 30 inches.

The disease develops on tree-ripe fruit during the winter and spring months. In the early stages, the dry, reddish-brown lesions are circular or oval in outline, but may rapidly extend over a large part of the surface of the fruit unless dry weather intervenes. The diseased tissue is depressed somewhat below the bottom of the normal oil glands into the white portion of the rind, but does not extend into the pulp. The line between the healthy and diseased tissue is distinct and sharply defined.

The disease is caused by *Pseudomonas citriputeale*, first described by C. O. Smith from Riverside, California, in 1913, under the name of "Black Pit of the Lemon." In 1924 the author recorded it from Australia.

Preliminary notes on avocado fruit decay. WM. T. HORNE.

Rhizopus nigricans Ehr. causes a wound decay of ripe fruit of avocado, and the mycelium is capable of slowly penetrating the uninjured surface. The seed is not injured. A fungus, probably *Diplodia natalensis* Ev., from Florida fruit, causes active dark decay and sometimes destruction of the seed with dry rot. *Colletotrichum glocosporioides* Penz. and *Pestalozzia* Sp. are conspicuous in decaying Florida fruits. California fruits decay mainly from wound infection with species of *Alternaria, Cladosporium, Fusarium*, and similar molds. The rots produced are slow-acting and rather dry. Bacteria of various types are abundant in late stages of decomposition, but apparently are not pioneers in tissue invasion.

The take-all disease in Canada. W. P. FRASER, P. M. SIMMONDS and R. C. RUSSELL.

In an extensive field survey made during 1923–25 the "take-all" disease of wheat was found causing considerable loss in a large number of spring wheat fields throughout the semi-wooded part of northeastern Saskatchewan, between latitudes 52 and 53 and as far north (25 miles) as was surveyed. "Take-all" was found also in north-

central Alberta. The disease was not observed in the prairie areas. "Take-all" occurred on the first crop after breaking, and was more severe on the second crop and in fields devoted to wheat a number of years. Mature perithecia of what is apparently *Ophiobolus cariceti* have been found on *Torresia odorata*. The mycelium of the same fungus was found on *Bromus* spp., growing in wheat fields. From observational evidence, it is believed that the "take-all" fungus is indigenous to this area, and parasitizes a number of the native grasses, particularly those with rhizomes. In the greenhouse, the Saskatchewan fungus attacked all of the members of the subspecies of *Triticum* tested. In addition to the susceptible grasses noted by Kirby, the following were attacked: *Avena Hookeri, Bromus inermis, B. latiglumis, B. Porteri, Agropyron dasystachyum* and *Elymus innovatus*.

The influence of carbon dioxide and oxygen on the growth of Ophiobolus graminis in pure culture. MURLEY FELLOWS.

By varying the CO_2 and oxygen content of the atmosphere it was found that *Ophiobolus graminis* grows in oxygen contents varying from 0.2 to 21.3 per cent in both the liquid and solid media. In the decoction, a gradual diminution occurred as the oxygen percentage was lowered. On agar, no marked diminution obtained until the oxygen was below 6 per cent.

A variation in the carbon-dioxide content between 0.9 and 18 per cent did not affect the growth of *Ophiobolus graminis* greatly. Eighteen per cent reduced the growth somewhat. In all the carbon-dioxide experiments, the growth curve was bimodal. The oxygen content of the various carbon-dioxide concentrations was kept near normal.

It is not believed that the variations in carbon dioxide and oxygen as found in arable soils are great enough to materially affect the growth of *Ophiobolus graminis*. (Investigations conducted by the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, with the Wisconsin and Kansas Agricultural Experiment Stations cooperating.)

The effect of tillage, fertilizers, and rotations on the spread of wheat foot-rot. LEO E. MELCHERS and M. C. SEWELL.

Two years' additional data on winter wheat tillage plots confirm the conclusion reached in a recent publication, namely, that shallow and late tillage checks the rapid spread of foot-rot, while deep, early plowing favors its survival and spread. Furthermore, plots, disced during the summer to keep the weeds down, showed only light infection; keeping the weeds scraped off without further stirring the soil, or merely discing at seeding showed no appearance of foot-rot. There appears to be an equal spread where, (1) stubble is burned before plowing, (2) where straw is applied and plowed under, (3) where straw is applied and disced under. Where straw was applied in winter as a top dressing, foot-rot spread uniformly over the entire area treated.

A series of fertilizer plots, devoted to wheat since 1919, with cowpeas as a green manure catch crop every third year, developed foot-rot in 1922. These plots receive various applications of phosphate, potash, nitrogen, and barnyard manure, and are usually plowed early and deep. The application of these various fertilizers seems to have had little influence on the spread of foot-rot during four years' observations. (Cooperative investigations by the Kansas Agricultural Experiment Station and the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.)

The influence of temperature upon the metabolism and expression of disease resistance in selfed lines of corn. JAMES G. DICKSON and JAMES R. HOLBERT.

Disease resistant and susceptible strains of Yellow Dent corn have been selected from selfed lines of corn and self-pollinated until practically homozygous. These strains have been studied comparatively under controlled environmental conditions as well as under varying field conditions. The resistant strains remained healthy over a much wider range of temperature than open-pollinated material or the susceptible strains as shown in the following table.

Strains of Corn	Temperature range of resistance to seedling blight in degrees C.
Resistant selfed lines	16–32
Selected open-pollinated	24–32
Susceptible selfed lines	32

The metabolism of the resistant, selfed strains, although similar to that occurring in the open-pollinated corn, is stable over a greater range of temperature. These resistant strains are high in hexose substances and low in pentosan materials at 16° C. and higher. In contrast, the open-pollinated corn maintains this high ratio of hexose substances at temperatures of 24° C. and higher.

The metabolism of the susceptible, selfed strains does not correspond to that of either the resistant strains or open-pollinated corn. The susceptible strains studied so far are different in composition and in relative ratio of substances present at all temperatures. In general, the pentosan substances increase with the rise in temperature rather than decrease as in the resistant strains.

(Cooperative investigations by the Wisconsin Agricultural Experiment Station, the Funk Brothers Seed Company, and the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.)

Seed treatments for the control of certain diseases of dent corn. JAMES R. HOLBERT, CHAS. S. REDDY and BENJAMIN KOEHLER.

Extensive experiments conducted at various places in Illinois during the past five years have shown that dent corn grown from seed infected with *Diplodia zeae* and *Gibberella saubinetii* is greatly improved in both yield and quality by seed treatment, while corn from *Fusarium moniliforme* infected and scutellum-rotted seed has not been improved. Only under certain soil conditions, not fully understood, have seed treatments increased the yield of corn grown from seed infected with *Cephalosporium acremonium*. Treated, infected seed has not yielded as well as untreated, nearly disease-free seed. Seed treatment may prove important when it is impossible to secure sufficient nearly disease-free seed. The summarized results follow.

Seed treatment materials	Total No. replications	Nearly disease-free seed		Diplodia-infected seed		Gibberella-infected seed	
		Incr.	Decr.	Incr.	Decr.	Incr.	Decr.
		Bu.	Bu.	Bu.	Bu.	Bu.	Bu.
Uspulun (0.5%)	317	—	0.5[a]	15.6	—	15.1	—
Bayer Comp. "	24	—	2.1[a]	19.3	—	7.0	—
Semesan "	72	—	0.5[a]	6.4	—	18.2	—
DuPont No. 12 "	72	—	3.8	14.0	—	—	—
Mercury A "	36	—	1.8[a]	16.4	—	19.8	—
Corona 620 (0.15%)	120	—	3.1[a]	21.7	—	17.6	—
Germisan "	80	—	1.7[a]	17.4	—	—	—
Standard Oil H_1 (100%)	42	0.2[a]	—	13.1	—	17.9	—
DuPont No. 12 (Dust)	42	5.1[a]	—	9.7	—	12.1	—
" " 18 (Dust)	24	—	3.7[a]	10.7	—	16.6	—
Semesan (Dust)	12	—	6.6[a]	9.6	—	—	—
Semesan, Jr. (Dust)	30	—	1.3[a]	20.7	—	19.0	—
Bayer Dust	30	8.5	—	15.8	—	28.9	—
Corona 640 S (Dust)	27	—	3.1[a]	8.5	—	19.8	—
S. F. A. No. 225 (Dust)	12	8.2	—	16.8	—	—	—

[a] Odds less than 30: 1, others greater.

(Cooperative investigations by the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, the Funk Brothers' Seed Company, and the Illinois Agricultural Experiment Station.)

The effect of the alternate hosts on physiologic forms. S. M. DIETZ.

The effect of *Rhamnus* species on the fixity of physiologic forms of *Puccinia coronata* was studied to determine the possible influence of these alternate hosts on the infective capabilities of the aecidiospores produced on them and the possible increase of their gramineous host range.

It previously has been shown that *Puccinia coronata avenae*, *P. coronata calamagrostis*, *P. coronata festucae*, and *P. coronata holci* will not produce normal uredinial infection *inter se*. However, aecidiospores from *Rhamnus caroliniana*, *R. cathartica*, *R. lanceolata*, *R. pinetorum*, *R. smithii* and *R. rubra*, produced by teleutospores from *Avena sativa*, formed normal infection on *Nothoholcus lanatus* under controlled greenhouse conditions. Aecidiospores from *Rhamnus lanceolata*, inoculated with teleutospores from *Calamagrostis canadensis*, produced abundant uredospores on *Avena sativa* in nine trials conducted over three years. Aecidia on *Rhamnus lanceolata* produced by teleutospores on *Avena sativa* formed abundant uredospores on *Calamagrostis canadensis*. Aecidia collected on *Rhamnus alnifolia* in the open produced abundant uredinial infection on *Avena sativa*.

Aecidiospores from *Rhamnus lanceolata*, produced by teleutospores from *Festuca elatior*, formed abundant uredospores on *Festuca interrupta*, *Hordeum murinum*, and *Phalaris stenoptera* and a lesser degree of infection on *Andropogon trinii*, *Bromus inermis*, *Lolium subtratum*, *Phalaris angusta*, *P. arundinaceae*, *P. bochmeri*, *P. brachystachys*, *P. bulbosa*, *P. nodosa*, *P. truncata*, and *Stipa* sp. The above recorded grasses produced no infection when repeatedly inoculated with uredospores of *Puccinia coronata*

festucae. (Cooperative investigations by the Iowa Agricultural Experiment Station and the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.)

Alternate hosts of Puccinia coronata II. S. M. DIETZ.

Rhamnus alpina, R. chlorophera, R. ilicifolia, R. infectoria, R. smithii, and *R. tinctoria* are susceptible to *Puccinia coronata festucae*. When inoculated with teleutospores of crown rust from *Festuca elatior, Rhamnus dahurica, R. dahurica* var. *nipponica, R. frangula*, and *R. japonica* became infected, but did not produce aecidia. *Rhamnus japonica* showed no infection with the above physiologic form. *Berchemia scandens* and *Zizyphus lycoides* showed pycnidial infection, while *Ceanothus americanus, Elaeagnus angustifolia, Lonicera flava*, and *Zizyphus obtusifolia* were copiously flecked by *Puccinia coronata festucae*. *Lonicera flava* also was markedly flecked by *Puccinia coronata calamagrostis*.

Local epidemics of crown rust were started again during the spring of 1925, in the Upper Mississippi Valley, by *Rhamnus cathartica* and *R. lanceolata*. The latter produced the heaviest aecidial infection during the past nine years in the spring of 1925.

One hundred and fifty-eight additional plantings of *Rhamnus cathartica*, 69 of which were urban and 89 rural, were located during 1925. These plantings contained 11,057 counted bushes besides 5,380 feet of hedge where the bushes were not counted. (Cooperative investigations by the Iowa Agricultural Expriment Station and the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.)

Physiologic specialization of Ustilago zeae and Puccinia sorghi and their relation to corn improvement. E. C. STAKMAN and J. J. CHRISTENSEN.

Ustilago zeae is one of the destructive pathogenes of corn. Apparently the only method of reducing losses appreciably is the production of resistant lines. However, there is circumstantial as well as direct evidence that there are several physiologic forms of the organism. This fact is likely to complicate the problem of developing resistant varieties. *Puccinia sorghi* is not considered a destructive pathogene. However, in 1925, it did considerable damage on certain selfed lines of corn in the plant breeding plots at the University of Minnesota. It is possible, therefore, that corn rust may become an important factor in corn production if susceptible sorts are produced and grown commonly. Some lines of a given variety of corn are extremely resistant, while others are highly susceptible. The rust situation is also complicated by the existence of several physiologic forms. At least three, and possibly five, have been recognized by their action on eight selfed lines of corn. A very virulent form was collected in Oklahoma, Kansas, Nebraska, and New Hampshire, while a less virulent one was obtained from several localities in Minnesota, and a very weak one from Texas. There is no correlation between rust resistance and smut resistance of selfed lines. (Cooperative investigations by the Section of Plant Pathology and the Section of Plant Breeding, Department of Agriculture, University of Minnesota.)

Physiologic specialization of Fusarium lini, Bolley. W. C. BROADFOOT and E. C. STAKMAN.

Fusarium lini consists of several physiologic forms. At least eight are distinguished by their parasitic action on the following varieties of flax: Primost, Minn. 25 (C. I. 177); Winona, Minn. 182 (C. I. 179); N. D. 3080 (C. I. 275); N. D. 40013 (C. I. 241). Six of the forms differ greatly from each other in virulence. Two are about equally virulent, except that one consistently causes loss of chlorophyll above the cotyledons in all varieties of flax inoculated. It also causes loss of chlorophyll in seedlings of barley, oats, and rye.

The cultural characteristics of some forms differ from those of others, but this is not a safe criterion for the determination of all of the forms. Neither can the forms be differentiated by rate of growth at different temperatures. There are differences in size and septation of spores of different forms grown under the same conditions, but there also are similar differences when the individual forms are grown under different conditions. Therefore the forms cannot be differentiated by spore morphology. Some of these forms infect heavily certain resistant varieties of flax, but it is not yet known how important this fact is in controlling flax wilt through the development of resistant strains.

A strain of sorghum kernel smut which infects milo and hegari. W. H. TISDALE, L. E. MELCHERS, and H. J. CLEMMER.

In comprehensive experiments with sorghums completed in 1921, milo, feterita, and hegari were found to be almost immune from kernel smut. In 1923 kernel smut was reported in milo in southwestern Kansas, northeastern New Mexico, and northwestern Texas. Milo and hegari were found to be smutted in this region in 1924. Experiments conducted cooperatively by the offices of Cereal Investigations and Dry-Land Agriculture, Bureau of Plant Industry, United States Department of Agriculture, and the Kansas Agricultural Experiment Station in 1924 and 1925 have shown that the smut from milo and hegari infects pure lines of milo and hegari and the sorghums susceptible to the commonly occurring strain of *Sphacelotheca sorghi*. It does not infect feterita. Covered kernel smut from the susceptible sorghums grown at Manhattan, Kansas, failed to produce smut in milo or hegari as in previous experiments. Infected heads of milo and hegari often are only partially smutted by this new strain of smut. The fact that a kernel smut now exists which attacks hitherto resistant varieties greatly complicates the problem of breeding for resistance. Although there are certain characters of the sori of this smut and the sterile cells of the fungus which resemble *S. cruenta*, it is more typical of *S. sorghi*.

A Pythium seedling blight and root rot of dent corn. HELEN JOHANN, J. R. HOLBERT, and JAMES G. DICKSON.

During the past few years an undetermined species of *Pythium* has been found associated with root rots of dent corn in Illinois and Wisconsin. It is somewhat closely related to the species causing root rot of cane, as described by Carpenter, in that it has lobulate sporangia and smooth oogonia and oospores.

Infection usually occurs at the tips of rootlets and proceeds proximally, producing a soft rot, involving first the cortex and later the vascular elements. Unlike infections by *Diplodia zeae* and *Gibberella saubinetii*, the mesocotyls are not attacked until roots are practically destroyed.

Soil temperatures of 12–16° C., and high soil moistures, tend to favor blighting which prevents germination. Some seedling blight may occur above 16° C. If the infection is not severe enough to kill the seedlings, they may be more or less retarded in size and vigor by the soft rot of the feeding roots.

Field inoculations at Bloomington, Illinois, with 18 pedigreed strains of corn in the cool, wet spring of 1924 seem to confirm these results. The average reduction in stand was 30 per cent. The maximum reduction in yield of sound corn per plant was 65 per cent in one strain, notwithstanding thinning due to a 31 per cent reduction in stand. (Cooperative investigations by the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, the Wisconsin Agricultural Experiment Station, and the Funk Brothers' Seed Company.)

Sclerospora graminicola on corn. I. E. MELHUS and FRANK VAN HALTERN.

The oospores of *Sclerospora graminicola* on *Setaria viridis* may cause seedling infection of corn, teosinte, sorghum, and millet. The symptoms produced on these hosts,

in general, resemble those on *Setaria viridis* in the open, except that oospores have not been observed on corn, teosinte, or sorghum. Conidial sporulation is confined to the early-seedling stage. This fact makes the disease difficult, for the present, to diagnose in the field on corn. Plants showing the characteristic etiolation, dwarfing, and striping were collected this past season on corn seedlings and more mature plants. Oospore infection has never been obtained after emergence of the seedling.

Yellow pearl popcorn inoculated in the greenhouse with oospores and transplanted to the field in the seedling stage showed 73 per cent infection. Sixty-five per cent of these died before maturity. The remaining plants were stunted, produced neither ears, ear shoots, nor normal tassels. Ninety-nine per cent of the inoculated checks lived.

The incubation period for oospore infection on *Setaria* was six days, teosinte eight, and corn ten. Oospore infection is prevailingly systemic. The oospores require no rest period. They may function immediately on liberation from the host tissues; yet they remain viable in the soil for at least two growing seasons. Oospores stored dry in the laboratory for 14 years were not viable.

Observations on the stem rot of rice caused by Sclerotium oryzae Catt. V. H. YOUNG.

Stem rot caused by *Sclerotium oryzae* is the most serious disease of rice in Arkansas. It is rather widespread and all commercial varieties are attacked. Observations on diseased fields, allowed to lie idle one season, indicate that the incidence of the disease has thereby been greatly decreased. Other hosts for the stem-rot fungus have not been found, but red rice, a weed variety, is susceptible and lives over, producing seed on unirrigated land and thus carrying the disease over the fallow period from one rice crop to the next. Diseased plants ordinarily do not appear in numbers until after the final drainage. However, diseased red rice has been found a month prior to harvest and the greatest losses observed were in fields containing such volunteer plants.

Infection of young plants was not observed, but seedling infections were secured with pure cultures *in vitro*. Soil inoculations at higher temperatures in soil temperature tanks did not result in earlier infection, nor was the *amount* of infection influenced by soil temperatures from 22.5° C. to 35° C. Soil from affected fields gave infection under greenhouse conditions. Growth of *Sclerotium oryzae* on rice stubbles after harvest results in considerable increase in the amount of over-wintering material.

Studies in rust resistance. (Invitation paper) E. B. MAINS.

Microscopical studies of rust resistance and susceptibility. (Invitation paper) RUTH F. ALLEN.

Smut resistance in cereals. (Invitation paper) GEORGE M. REED.

Susceptibility of wheat varieties and selections to loose smut. F. D. FROMME.

Differences in incidence of loose smut in the Stoner and Leap varieties of wheat are shown to be due to differences in susceptibility. Inoculation of florets of the two varieties produced 62 per cent of infected plants of Stoner and only 3 per cent of infected plants of Leap. Thirty-five per cent of Stoner heads were smutted as compared with 4 per cent of heads of Leap.

Other wheat varieties grown under test conditions during several seasons showed marked differences in incidence of loose smut and each variety, with few exceptions, showed a high degree of uniformity when rated in terms of a standard. Similar differences, but of greater degree, are found in pure line selections of Fulcaster. Some are very susceptible, some moderately so, and some are highly resistant. The most sus-

ceptible selection is eight times as susceptible as the Fulcaster parent and the most resistant is twenty times as resistant as Fulcaster. This variability is possibly related to the hybrid origin of the variety. The probability of developing a highly resistant strain of Fulcaster through pure line selection is indicated.

Breeding and selecting for resistance to corn root-rot. (Invitation paper) G. N. HOFFER.

Inheritance of immunity from Melampsora lini. A. W. HENRY.

Crosses were made between immune and susceptible varieties of *Linum usitatissimum*. Three immune parents were used; a selection from a lot of commercial Argentine flax; Bombay (C. I. 42); and Ottawa 770B. The F_1 plants of all crosses were immune. The segregation in the F_2 of a cross between the Argentine selection and Saginaw, a susceptible fiber variety, approximated a ratio of 15 immune plants to 1 susceptible plant. The F_2 of a cross between Bombay and Winona, a susceptible seed flax, showed a simple monohybrid segregation. Apparently there was a single factor difference between immunity and susceptibility in a cross between Ottawa 770B and Saginaw. The former has white flowers and yellow seeds, and the latter blue flowers and brown seeds. Blue flowers and brown seeds were dominant, and a ratio of 3 blue-flowered, brown-seeded plants to 1 white-flowered, yellow-seeded plant was obtained in the F_2. These characters apparently are linked and were inherited independently of immunity from rust, so that in the F_2 a segregation approximating a 9: 3: 3: 1 ratio was obtained. (Cooperative investigation by the Minnesota Agricultural Experiment Station and the Office of Cereal Investigations and Fiber Plant Investigations, U. S. Department of Agriculture.)

Seed potato improvement work. (Invitation paper) WILLIAM STUART.

Studies upon the inheritance of Fusarium-resistance in cabbage. J. C. WALKER.

By selfing individual cabbage plants through three generations, a line has been established which is apparently homozygous for resistance to *Fusarium conglutinans* under such field conditions as favor development of the yellows disease. Similarly, through selfing and close-pollination, individuals, apparently homozygous for susceptibility under the same environment, have been selected from commercial varieties. The F_1 hybrids from several crosses between resistant and susceptible individuals were nearly all disease-free. A total of 1,135 F_2 hybrid plants were grown in 1925 on infested soil; 843 were completely free from yellows, 292 were diseased. Segregation was therefore close to a ratio of three resistant to one susceptible. Several F_1 plants were crossed with a susceptible individual. Of these F_2 hybrids, 151 plants were grown in infested soil; 77 were completely resistant and 74 were diseased. Segregation was close to the 1: 1 ratio. These data indicate that resistance to yellows, as expressed under field environment, is a dominant unit-character. (Cooperative investigations between the Office of Cotton, Truck and Forage Crop Disease Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, and the Agricultural Experiment Station of the University of Wisconsin.)

Resistance in sugar beets to curly-top. EUBANKS CARSNER.

A large number of strains of sugar beets selected for their apparent resistance to curly-top, together with 16 strains selected only for desirable agronomic characters, were subjected to a rigorous test in a leafhopper (*Eutettix tenella*) infested locality. In addition to the usually great natural infection hazard, viruliferous leafhoppers were caged on each plant. Control plats of commercial seed were similarly treated.

The results showed that among the strains which had been selected without regard for resistance, some were more susceptible than the controls, while others, especially one strain, were less affected than the controls. Among the strains which had been selected for resistance several were definitely less injured than the controls—one of them conspicuously so.

A comparatively large planting of the first generation progeny of a mass selection for resistance also suffered less from the disease than did the commercial parent strain. The results of the work thus far, especially those of the past season, establish the fact that there are marked variations in resistance to curly-top among sugar beets, and seem to justify the belief that a satisfactorily resistant commercial strain can be developed.